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INDEX AUCTORUM.

From the Pharmacological Department of the Caroline Institute,
Stockholm.

On the Influence of the Pulse Amplitude on the Carotid Sinus Pressure Reflex.

By

ERNST BÁRÁNY.

(Received 8 November 1941.)

As is well known, experimental changes in the mean pressure within the carotid sinus are followed by reflex blood pressure changes. It does not follow, however, that the mean pressure is the adequate stimulus of the carotid sinus, the magnitude of the depressor effect released from the sinus being determined solely by the mean pressure in the latter. There remains the possibility, that the amplitude and frequency of the pulsations in the sinus have an influence on the reflex. In the course of a still unfinished investigation on kindred problems some experiments pertaining on this question were performed in 1938. They are now published separately because E. STRAUSS (1940) recently has published similar experiments with different results.

Rabbits in urethane narcosis were used. A concentric double cannula was inserted in the common carotid on one side and the vessels leading from the bifurcation carefully isolated and ligated. As perfusion fluid 50 % defibrinated ox blood in Tyrode, kept 24 hours in the refrigerator before the experiment, was used. A special perfusing system allowing independent control of perfusion speed, intrasinusoidal mean pressure and pulse amplitude was devised. For description see fig. 1. The pump for generating the pulsations gave approximately sinusoidal pressure variations, the speed of which were adjusted to 120 strokes per min. It would have been desirable to measure the actual pulse amplitude in the preparation. Because of the extremely small volume of the preparation, only a practically isometric manometer could have been used. As no such instrument was available the maximal pulse amplitude was adjusted so as to give considerably more violent pulsatile

movements of the carotid sinus bag than those performed by the contralateral carotid sinus while still in connection with the circulation of the animal. Then, the cardioaortic nerves and the contralateral sinus caroticus were destroyed and blood pressure recorded in the contralateral common carotid.

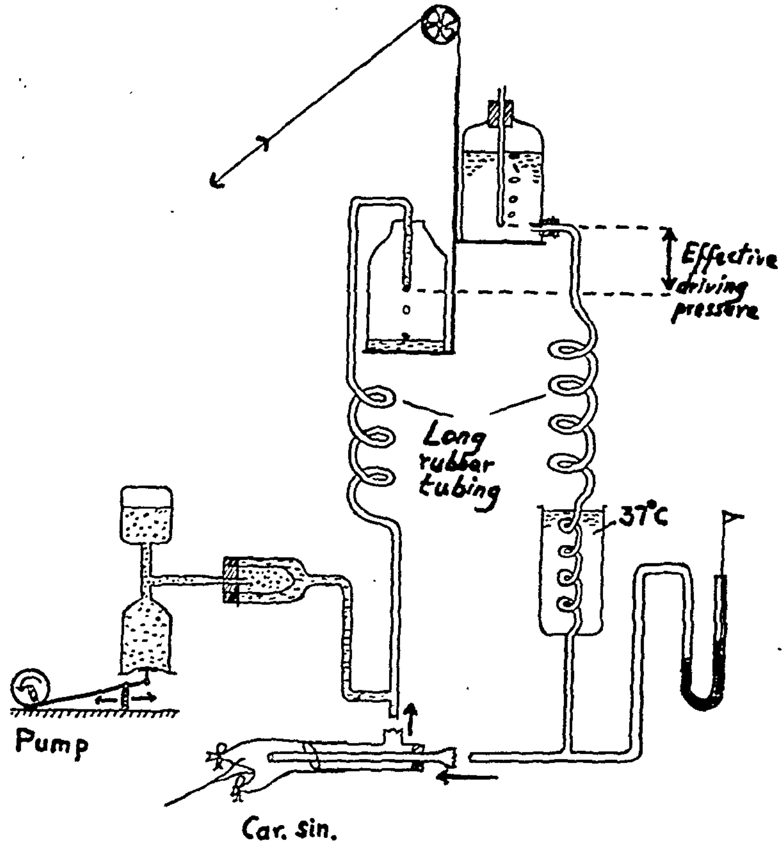

Fig. 1. Perfusing system.

Mean pressure is determined by the height of the two flasks suspended from the ceiling, rate of perfusion by the effective driving pressure as indicated, rate and amplitude of pulsations by adjustments on the pump. The long rubber tubings act as mechanical chokes, preventing the pulsations from being short-circuited by the leads for perfusion fluid. The air chamber at the pump serves to reduce harmonics in the pulses. The rubber membrane separating the fluid in the pump from that in the system is a rubber glove finger.

The main experiment was performed in the following manner: The stroke volume of the pump was set to 0, the intrasinusoidal pressure adjusted to about 40 mm Hg, blood pressure allowed to equilibrate and the intrasinusoidal pressure raised. A drop in blood pressure showed, that the preparation worked. After equilibration of the blood pressure on the new level pulsations

were introduced by adjusting the stroke volume of the pump to the predetermined level. After a minute or so the pulsations were removed and the pressure in the preparation reduced in order to test the blood pressure reflex. One of about a dozen experiments at different pressure levels but with identical results is shown in fig. 2. It was never possible to observe even the slightest effect of even very violent pulsations.

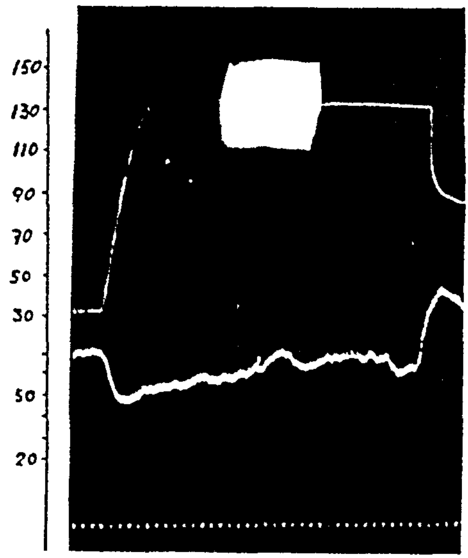

Fig. 2.

Upper curve: intrasinusoidal pressure.
Lower curve: blood pressure.
Time: 10″.

Discussion.

The results obtained differ from those of E. STRAUSS (1940) who found that pulsations increased the depressor effect. He used vagotomized dogs and a different perfusion system, the pulse curve of which certainly was not sinusoidal. It might be that this is the explanation of the discrepancy between our results. An other difference between our experiments is that STRAUSS did not make a quick change from pulsations to no pulsations but recorded the whole "Blutdruck-Charakteristik" (E. KOCH 1931) with the different types of stimuli. Therefore, his preparation could have changed between the different runs. No checks of this point are mentioned.

The negative result obtained in the above experiments is somewhat surprising and one wonders if the symmetrical form of the pulsations could have anything to do with it. The central pulse certainly is quite asymmetric, with a steep rise and a slower fall of pressure. It might be that the steep rise releases an extra volley of activity which is not compensated for by the silent period during the fall. With sinusoidal pressure variations this might not be the case. Experiments with different pulse forms and recording of action potentials would be desirable.

Summary.

Superposition of sinusoidal pulsations upon a steady mean pressure in the isolated carotid sinus has no influence on the blood pressure.

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Aus der Physiologischen Abteilung, Karolinska Institutet, Stockholm.

Die Einwirkung von Blutdrucksänderungen im Carotissinus auf die Atmung bei der Katze.

Von

H. BJURSTEDT und C. M. HESSER.

(Eingereicht am 15. Dezember 1941.)

Es ist schon lange Zeit bekannt, dass die Ventilation bei Verschluss der beiden Carotiden grösser wird; diese Erscheinung hat sich als sehr konstant und leicht zu demonstrieren erwiesen. Nur hat man den Befund nicht auf einen allgemein anerkannten Mechanismus zurückführen können. Früher wurde die zunehmende Ventilation allein als Erfolg der verschlechterten zentralen Durchblutung angesehen, auch wenn SICILIANO 1900 anführte, dass nach Verschluss der beiden Vertebrales, die vielmehr für die Blutversorgung des Atemzentrums verantwortlich sind, die Änderung der Ventilation kleiner als nach Verschluss der Carotiden wäre. Spätere planmässige Untersuchungen an isolierten Carotissinuspräparaten seit der Entdeckung der Sinusreflexe haben gezeigt, dass die Vergrösserung der Ventilation grösstenteils auf Einwirkung der Druckabnahme im reflexogenen Gebiet der Carotiden beruht. Da nach der Entnervung beider Sinus keine Änderung der Ventilation nach Änderung des endosinualen Druckes vorhanden war, wurde die oben genannte Vergrösserung als Folge des Wegfalls einer reflektorischen Hemmung aufgefasst (HEYMANS und BOUCKAERT, 1930, KOCH und MARK, 1931).

Den Barorezeptoren, von denen nach den genannten Verfassern diese tonischen Impulse zentralwärts ausgehen sollten, wurden also neben der »blutdruckzügelnden» Wirksamkeit eine gleichzeitig

atmungsregulierende Rolle zuerkannt. KOCH und MARK haben aber hervorgehoben, dass bei Änderung des endosinualen Druckes Atmungsänderung bei unverändertem allgemeinem Blutdruck gelegentlich beobachtet werden konnte, was die Beteiligung der Barorezeptoren in Frage setzt. SCHMIDT (1932) vermutet, dass die Einwirkung von Blutdrucksänderungen im Carotissinus auf die Atmung wahrscheinlich anderen Ursprungs als die auf den Kreislauf wäre, da die Intensitäten der beiden Erscheinungen von einander oft völlig unabhängig sind. EULER und LILJESTRAND (1937) lieferten Stütze für die Auffassung, dass die Ventilationsvergrösserung bei Senkung des endosinualen Druckes in Perfusionsversuchen an Hunden viel mehr auf Reizung der Chemorezeptoren als auf Wegfall einer pressorischen Hemmung zurückzuführen wäre. Eine Senkung des Sinusdruckes durch Verschluss der Carotiden hat ferner eine Frequenzsteigerung der in den Sinusnerven der Katze verlaufenden Impulse zufolge; diese kann aber durch Hyperventilation mit Sauerstoff aufgehoben werden und dürfte somit von der Erregung der Chemorezeptoren abhängig sein (EULER, LILJESTRAND und ZOTTERMAN, 1939). Weitere Beobachtungen von EULER und LILJESTRAND 1940 haben gezeigt, dass bei Perfusion der Carotiden mit sauerstoffarmem Blut (Sättigung = 33 %), im Gegensatz zu sauerstoffreichem, eine Senkung des endosinualen Druckes keinen Effekt auf die Atmung hat, da die Chemorezeptoren in diesem Fall wahrscheinlich bereits bei hohem Perfusionsdruck durch Sauerstoffmangel maximal gereizt sind. Auch RUDBERG (1938) fasste die Ventilationsvergrösserung bei Verschluss der Carotiden als Folge einer lokalen Hypoxiereizung der Chemorezeptoren auf, da dieselbe bei niedrigem allgemeinen Blutdruck grösser war als bei gutem Druck (1938) und durch Sauerstoffatmung entgegengewirkt werden konnte (1940). Nach einem Versuch durch Embolisierung des *Glomus caroticum* mit einer *Lycopodium*-Suspension die Chemorezeptoren auszuschalten fand WINDER (1938), dass Respirationsreflexe bei endosinualen Druckänderungen noch auftraten. MARRI und HAUSS (1939) haben eine Untersuchung über die Einwirkung gewisser Faktoren auf die Atmungsreflexe bei endosinualen Druckvariationen ausgeführt, wobei eine Methodik benutzt wurde, die LIM und CHANG (1936) und HEYMANS, DONATELLI und SHEN (1938) beschrieben haben. Ein Gummi- oder umgestülpter Venensack wurde in den Sinus eingeführt, wobei die Absicht war, bei Druckänderung im Sacke einen pressorischen

Reflex ohne Beteiligung eines chemischen zu erhalten. Eine Steigerung bzw. Minderung der Ventilation bei Senkung und Erhöhung des endosinualen Druckes nach diesem Verfahren wurde demonstriert. Indessen dürfte das Fehlen einer Steigerung der Ventilation bei Senkung des endosinualen Druckes unter Inhalation von sauerstoffarmer Luft (MARRI und HAUSS, Abb. 1 C), das auf zentrale Depression zurückgeführt wurde, auch so erklärt werden können, dass die von hypoxämischem Körperblut gereizten Chemorezeptoren schon eine Ventilationsvergrösserung bewirkt hatten unter Verdrängung des Hemmungseffektes. Auch in einer Arbeit über vasomotorische Einwirkungen des Sinusdruckreflexes von GRIMSON und SHEN (1939, Abb. 1), wo dieselbe Methodik benutzt wurde, können keine pressorischen Atmungsreflexe bei wirksamen Barorezeptoren beobachtet werden (bei erhaltenen Vago-Depressoren). Die A. carotis communis der anderen Seite war in diesem Falle verschlossen worden, was an sich eine gewisse Hyperventilation herbeigeführt haben dürfte.

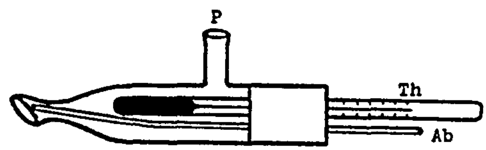

Abb. 1. Carotissinus-Kanüle.
P = Perfusionsblut. Th = Thermometer.
Ab = Abflussrohr.

Die folgende Mitteilung berichtet über Untersuchungen über die Bedeutung der beiden Arten von Rezeptoren im Carotissinus und zwar der Barorezeptoren für die Atmung bei der Katze.

Versuchsanordnung.

Als Versuchstiere wurden 12 Katzen verwendet. Nach vorheriger Anästhesierung mit Äther wurden die Tiere durch intravenöse Zufuhr einer 1 proz. Chloraloselösung (c:a 6 ml/kg Körpergewicht) anästhesiert. Die beiden Carotiden wurden im Bifurkationsgebiet schonend hervorpräpariert. Nur die grösseren Äste des Sinusgebietes wurden in einigem Abstand von der Bifurkation ligiert, damit die übrigen vom Gebiete ausgehenden kleineren Gefässe als Abfluss eines für die Versorgung der Rezeptoren während der Vorbereitungen des Versuches genügenden Blutstromes dienen könnten. 5 mg Heparin pro Kilo Körpergewicht wurden dann intravenös injiziert, um von Anfang an eine Koagulation in den abführenden Gefässen der Sinusgegend und in den Gefässen des Glomus caroticum zu verhindern. Spezielle Kanülen (Abb. 1), mit engen Abflussrohren versehen, wurden nach Unterbindung der Aa. carotides communes in die Sinussäcke eingeführt, so dass die kontinuierliche Zufuhr von neuem, sauerstoffgesättigtem Perfusionsblut auch

bei niedrigen endosinualen Drucken sichergestellt und kontro. .c werden konnte. Die chemische Einwirkung eines Rückflusses von Körperblut durch arterielle Anastomosen mit den Vertebralarterien wurde in dieser Weise weitgehend ausgeschaltet. Die Perfusion wurde mit völlig sauerstoffgesättigtem, heparinisiertem (1 mg/10 ml) Menschen- oder Schweineblut ausgeführt. Die Temperatur des zu perfundierenden Blutes wurde bei c:a 38° C gehalten. Der Arteriendruck, in der A. femoralis gemessen, und der endosinuale Druck wurden von Quecksilbermanometern registriert. Die Ventilation wurde quantitativ mit Hilfe eines Körperplethysmographen registriert.

Ergebnisse.

Mit Hilfe der obengenannten Versuchsanordnungen konnte eine fast ganz physiologische Durchströmung der vom übrigen Kreislauf isolierten Karotisgebiete mit einer Blutflüssigkeit von konstanter chemischer Zusammensetzung ausgeführt werden.

Es ergab sich bereits aus den ersten Versuchen, dass eine Änderung der Ventilation bei endosinualer Druckänderung gar kein

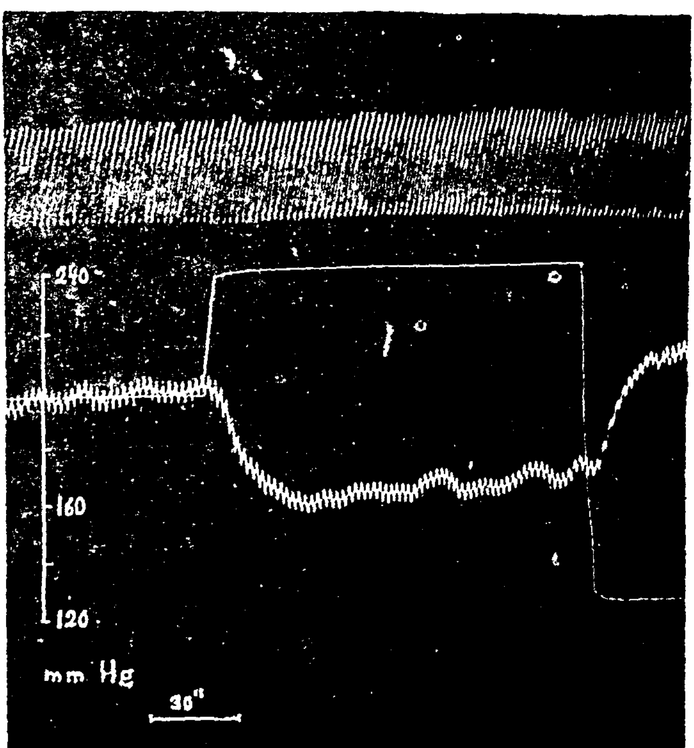

Abb. 2. Katze, Chloralose. Perfusion der beiden Sinusgebiete mit sauerstoffgesättigtem Schweineblut.

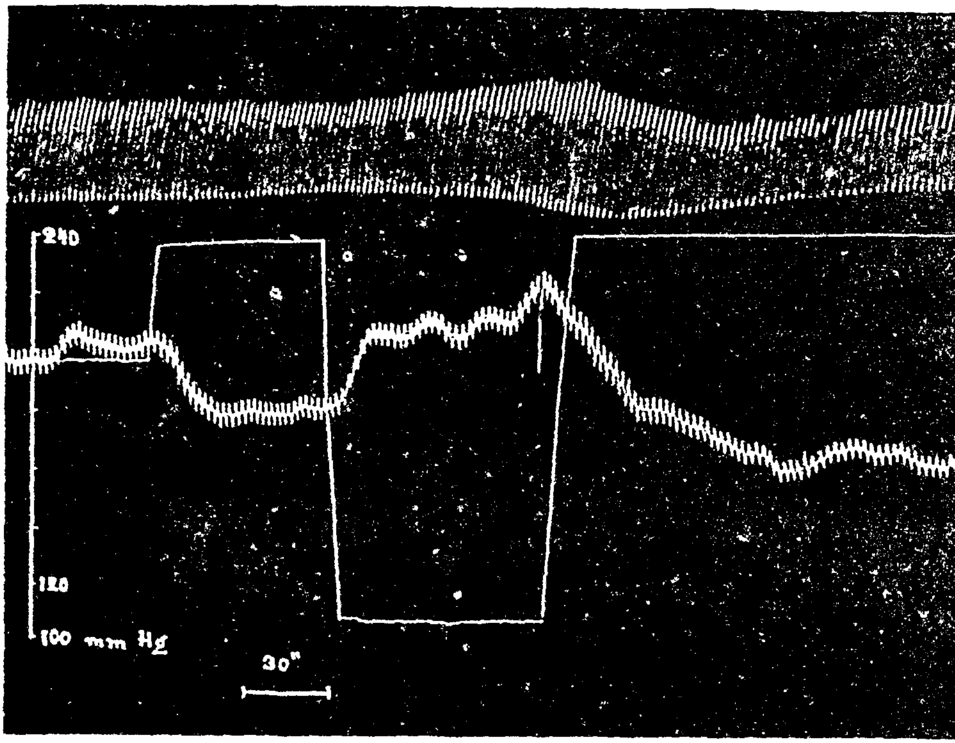

Abb. 3. Katze, Chloralose. Perfusion als in Abb. 2.

konstanter Befund ist. Bei Druckoszillationen innerhalb physiologischer Grenzen blieb meistens eine unveränderte Ventilation die Regel. Nur bei grossen Druckänderungen konnten Atmungsänderungen beobachtet werden, obwohl sehr oft auch dann die Atmung unverändert blieb. Die Barorezeptoren konnten bei keiner von diesen Gelegenheiten geschädigt oder gelähmt gewesen sein, da ihre reflektorische Einwirkung auf den Arteriendruck sehr schön beobachtet werden konnte (Abb. 2). Die Chemorezeptoren waren bei diesen Versuchen völlig funktionsfähig. Die bei den grösseren endosinualen Druckänderungen vorkommenden Fälle von Atmungsvariationen hatten nicht den Charakter eines unmittelbar einsetzenden Reflexmechanismus, wie man es erwarten würde, wenn es sich um eine zentrale Irradiation oder direkte Einwirkung pressorischer Impulse handelte (Abb. 3).

Bei unseren Fällen von Ventilationsänderungen war vielmehr ein allmähliches Eintreten der Ventilationsvergrösserung nach Senkung des endosinualen Druckes die Regel. Bei Erhöhung desselben nach vorherigem niedrigem Drucke konnte dagegen oft eine relativ schnell einsetzende Minderung der Atmung

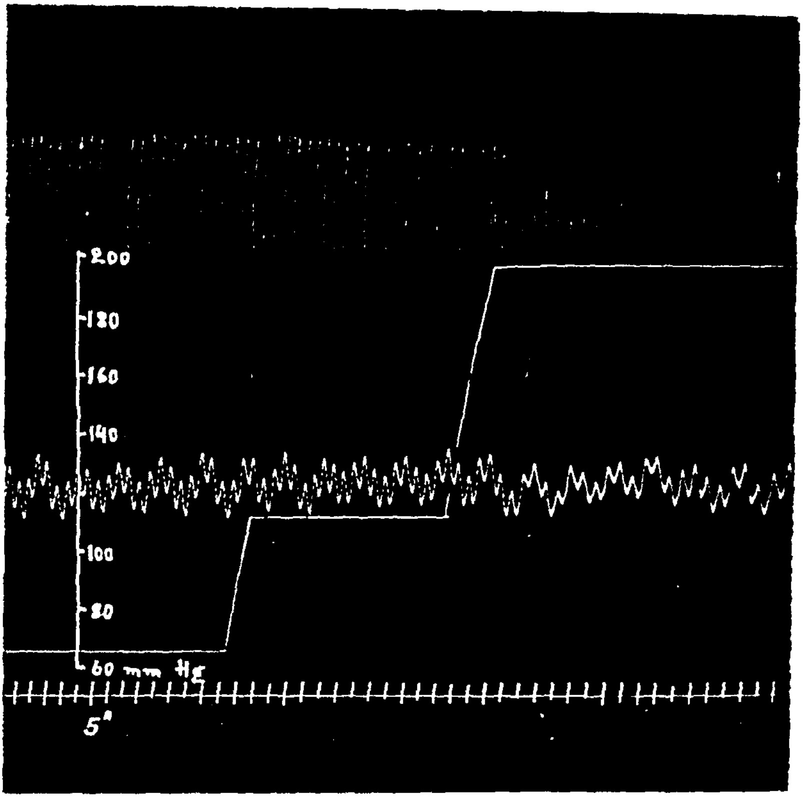

Abb. 4. Katze, Chloralose. Perfusion bei ausgeschaltetem Blutdrucksreflex.

beobachtet werden (Abb. 4). Atmungsveränderungen desselben Typus sind auch in der Arbeit von MARRI und HAUSS illustriert (MARRI und HAUSS, Abb. 4). Der Unterschied zwischen den beiden Erscheinungen ist deutlich und spricht gegen einen mechanischen Druckreflex, wo man gleichartige, schnell einsetzende Effekte erwarten würde. Der Befund lässt vielmehr vermuten, dass der niedrige Sinusdruck durch ungenügende Blutversorgung des Glomus eine lokal einsetzende Hypoxie und Ansammlung saurer Stoffwechselprodukte herbeiführt, die zur Erregung der Chemorezeptoren und damit der Atmung führen, und dass eine Druckerhöhung im Sinus die Ursachen dieser Erregung relativ schnell durch Fortspülen der sauren Metaboliten beseitigt. Die von KOCH (1930) bei endosinualer Druckerhöhung beobachtete Apnoe dürfte teilweise als Folge eines plötzlichen Wegfalls der zu Hyperpnoe führenden, chemischen Reflexe entstanden sein.

Während des Verlaufs unserer Versuche blieben die chemischen Atmungsreflexe gewöhnlich länger als die pressorischen Blut-

drucksreflexe bestehen. Es konnten somit leicht Präparate mit ausgeschalteten Barorezeptoren bei wirksamen Chemorezeptoren erhalten werden. Abb. 4 zeigt das Vorhandensein einer Ventilationsänderung bei endosinualer Druckänderung und geschädigten Barorezeptoren. Vor der Lähmung der Barorezeptoren konnten bei adäquater Reizung derselben in diesem Falle die gewöhnlichen Blutdruckseffekte hervorgerufen werden.

Aus Abb. 4 geht auch hervor, dass ein höherer endosinualer Druck als der Systemdruck notwendig ist, um diese Ventilationsminderung hervorzubringen, was wahrscheinlich auf einer erst dann eintretenden effektiven Durchströmung beruht.

In allen diesen Versuchen waren die Vago-Depressoren erhalten. Eine kompensatorische Wirkung der Aortarezeptoren dürfte wegen der mässigen Variationen im systemischen Blutdruck als Folge der oft weit grösseren Sinusdruckänderungen die fehlende Atmungswirkung nicht erklären können. Ferner waren die Aortarezeptoren nicht imstande die vom Sinus ausgelösten Blutdrucksänderungen zu kompensieren, weshalb es nicht anzunehmen ist, dass eine eventuelle Atmungshemmung von Sinus aus durch den Aorta-Mechanismus kompensiert werden kann. Wenn andererseits die Vago-Depressoren durchschnitten waren, trat eine presso-reflektorische Atmungshemmung ein, wie sie beim Hund mehrmals beschrieben worden ist.

Zusammenfassung.

Mit Hilfe der beschriebenen Versuchsanordnungen sind die folgenden Beobachtungen an Atmungsreflexen bei endosinualen Druckvariationen bei der Katze gemacht worden:

1) Endosinuale Druckänderungen brauchen keinerlei Veränderung der Atmung herbeizuführen, selbst wenn typische Blutdrucksreflexe auftreten.

2) Unter gewissen Bedingungen können endosinuale Druckänderungen Atmungsvariationen herbeiführen, auch wenn keine Blutdrucksreflexe auslösbar sind.

3) Die obengenannten Versuchsergebnisse liefern keine Stütze für die Annahme, dass Atmungsreaktionen bei endosinualen Druckänderungen bei der Katze über die Barorezeptoren unter normalen Bedingungen erfolgen, sondern stehen vielmehr im Einklang mit der Auffassung, dass die Atmungsveränderungen über die Chemorezeptoren ausgelöst werden.

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Nachtrag zur Korrektur: In Anlehnung an späteren Versuchen von BJURSTEDT und EULER (Acta Physiol. Scand. 1942, im Druck) wurden einige weitere Versuche angestellt, wo der allgemeine Blutdruck mittels einer Kompensationsvorrichtung variiert werden konnte. An Versuchstieren mit erhaltenen Vago-Depressoren und Sinusnerven trat z. B. bei Senkung und Erhöhung des normalen Druckes um 40—50 mm Hg keine signifikative Änderung der Atmung ein.

From the Physiological Department, Karolinska Institutet,
Stockholm.

Action Potentials from the Baroceptive and Chemoceptive Fibres in the Carotid Sinus Nerve of the Dog.

By

U. S. v. EULER and Y. ZOTTERMAN.

(Received 11 February 1942.)

According to SCHMIDT and COMROE (1940), several groups of workers have tried to record »chemical» potentials from the sinus nerve in the dog, but hitherto all experiments in that direction have failed. These negative results with dogs, in contrast to the positive results obtained with cats by BOGUE and STELLA (1934—35), ZOTTERMAN (1935), SAMAAN and STELLA (1935), and EULER, LILJESTRAND and ZOTTERMAN (1939), have led SCHMIDT and COMROE to express some doubts as to whether the »chemical» potentials recorded in the cat's sinus nerve are actually set up in the chemoreceptors of the carotid body. Although they realise that the negative result of the experiments on dogs may only mean that the potentials set up from the chemoreceptors are smaller in the dog than in the cat, they point out that in the cat there are »quite direct communications between the sinus nerve system and the superior cervical ganglion and that nerve impulses in the (thoracic) sympathetic are known to be increased by asphyxia, decreased by over-ventilation and increase in blood flow». They thus raise the question whether the potentials assumed to represent chemical impulses in the cat may derive from impulses originating from the sympathetic. SCHMIDT and COMROE do not give any details as to how they conceive that these connections might bring the electrical changes actually observed in the cat. As far as we can

see, there are only three ways in which these connections could be responsible for the chemical potentials recorded in the cat: 1) that preganglionic fibres run to the carotid body, where they relay; 2) that postganglionic fibres pass from the superior cervical ganglion through the carotid body further up in the sinus nerve; 3) that the »chemical» potentials recorded in the cat do not derive from the sinus nerve itself, but are due to electric leakage from the cervical ganglion or its connections.

As far as the two first alternatives are concerned, the potentials would derive from non-medullated fibres, while the potentials recorded in *the cat* obviously derive from medullated fibres. Further, we have found that all the medullated fibres running in the cat's sinus nerve degenerate peripherally to the seat of ligation (EULER, LILJESTRAND and ZOTTERMAN 1941 b). The third alternative is ruled out by the usual control, killing the nerve below the electrodes, which always abolishes all electric activity in the sinus nerve. In order to eliminate any discussion, we have cut all connections and removed the superior cervical ganglion in the cat. This procedure had no effect whatever on the electrical activity elicited by the natural stimuli to the chemoreceptors of the carotid body. On the other hand; it is conceivable that the chemoceptive impulses, in analogy to the baroceptive impulses (cf SCHWEITZER 1935), may induce changes in the activity of the sympathetic system of the kind observed by BRONK and his collaborators.

In a subsequent review, SCHMIDT and COMROE (1941) have come to suspect that the chemical potentials recorded by us in the cat might have been confused with small potentials originating from pressor receptors in the carotid sinus. This objection is easily dismissed by the observations made by BOGUE and STELLA (1934—35), as well by ourselves, that the chemical potentials can be elicited even when the pressure is lowered. Thus occlusion of the common carotid artery in the cat increases the frequency of these potentials. Further, our records from the cat show that, whereas the small potentials increase in number at each systole, the chemical potentials are evenly distributed (cf EULER, LILJESTRAND and ZOTTERMAN (1941 b fig. 4.). Thus we maintain that the chemical potentials recorded by us from the sinus nerve in the cat must, beyond any reasonable doubt, be taken to represent the impulses set up in the afferent fibres running from the chemoreceptors of the carotid body.

As it appeared, however, that the primary reason for SCHMIDT and COMROE's objections to our observations on the cat was the failure to record any action potentials in the dog's sinus nerve due to chemoreceptor activity, we decided to extend our studies of the chemical potentials to the sinus nerve in the dog.

Methods.

16 dogs in all, of weights from 6 to 25 kg were used. The animals were anesthetised with 0.1 g chloralose per kg intravenously. In most cases urethane or barbiturates had also to be given, in order to prevent shivering. The sinus nerve was prepared and freed of its sheath on a portion sufficiently long to permit of the application of electrodes, consisting of cotton wool wicks soaked in Ringer solution, and connected up with chlorinated silver wires. The action potentials were led to a resistance capacity coupled amplifier previously described (ZOTTERMAN 1936 and 1939), and a cathode ray oscillograph.

Blood pressure was recorded by means of an elastic manometer connected with the femoral artery. Intravenous injections were made through the femoral vein.

In a few experiments the external carotid was ligated just above the bifurcation, in order to separate the pressoreceptor area in the sinus proper and the chemoreceptor region of the glomus, the latter receiving its blood supply from the occipital artery. By tying the internal carotid and the pharyngeal artery and emptying the common carotid, we hoped to abolish the baroceptive impulses; but though these were greatly reduced in number there was still a considerable number of them left. When the sinus region was kept under normal circulatory conditions, it became necessary to compensate for the variations in the blood pressure accompanying the injection of drugs, notably lobeline. This was achieved by a compensatory device, consisting of a pressure bottle containing heparinized Ringer solution connected to the femoral artery and to a mercury valve and an oxygen pressure cylinder. In this way it was also possible to keep the mean blood pressure at a convenient level in respect to the intensity of the baroceptor activity, which, during normal blood pressure, would practically exclude detailed observations on the chemical activity in the dog.

In order to study the influence of purely local administrations of various solutions on the chemoreceptors, we have prefused the glomus region with heparinized arterial dog's blood under constant pressure from a pressure bottle. All branches from the bifurcation, except the occipital artery, were tied, and the perfusion cannula inserted in the common carotid. The occipital artery, peripheral to the glomus, was kept open, in order to ensure a good flow. Usually a pressure of at least 100 mm Hg was necessary to make the blood flow at a suitable rate from the pressure bottle into the artery. This is in accord with the observations of WINDER (1938) and shows that occlusion of the common carotid in the dog is not necessarily followed by a low pressure

in the glomus region or, presumably, an inadequate circulation, even if the internal carotid is ligated. In the perfusion experiments, and when the blood pressure regulating device was used, the animal was given heparine intravenously in doses of 5 mg pr kg body weight.

Results.

At ordinary blood-pressure, the sinus electroneurogram in the dog shows a massive volley of larger potentials at each systole and also a great number of medium-sized and small potentials, occurring all the time between the pulse waves. These latter potentials could not be abolished by over-ventilation with air or pure oxygen, nor could we observe any increase in their frequency when the dog was asphyxiated or over-ventilated with gas mixtures low in oxygen or high in carbon dioxide, as long as the pressure was kept constant. Even after ligating the external carotid between the bifurcation and the occipital artery, which greatly reduced the number of the pressure potentials, hypoxemia or hypercapnia did not give rise to any definite change in the electrical response. The most likely explanation of this was that the chemical potentials must be very much smaller in the dog than in the cat. We therefore cut down the nerve into thin filaments and led off from these, in order to increase the signal-to-noise ratio for the chemical fibres (see ZOTTERMAN 1936 and 1939). Even so, however, we did not succeed. This strengthened our suspicions that the chemical fibres in the dog range down to the C-class, according to ERLANGER and GASSER's nomenclature. Before abandoning the matter, however, we thought it worth while to test the effect of some of the most powerful stimuli to the chemical fibres which are known to produce very strong respiratory action, i. e. cyanide and lobeline. In order to reduce the number of the intersystolic pressure potentials, the blood pressure was kept at 85 mm Hg, with the aid of the compensatory device as described under "Methods", and this freed the response from visible pressure impulses in the interval between the pulse waves (fig. 1 A). Now, an intravenous injection of a small amount of cyanide gave rise to a change in the picture as seen in the record (fig. 1 B). The gap between the volleys of pressure potentials elicited by each pulse wave is now filled with small potentials. It gives the picture that might be expected from a mass response of very thin fibres, and is obviously the result of a summation of very small axone potentials, which would otherwise drown in the basal noise.

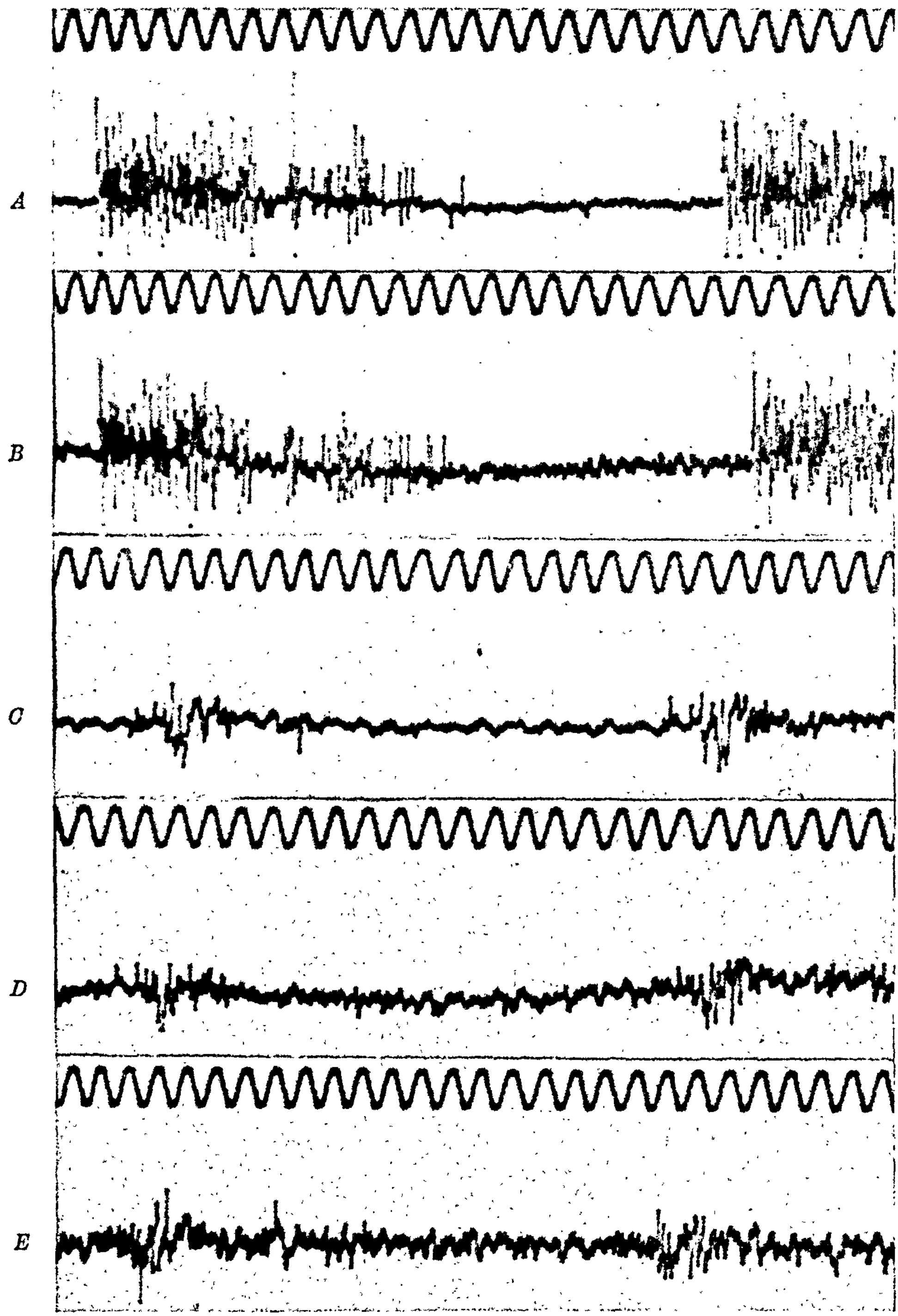


Fig. 1. Dog, chloralose. Action potentials from the sinus nerve. Blood pressure kept constant at 85 mm Hg. *A.* before and *B.* after an intravenous injection of $^1/_4$ ml of a weak KCN solution. *C.—E.* Records from a fine filament of the same sinus nerve. *C.* before and *D.* after an intravenous injection of 1.5 mg lobeline, *E.* after injection of KCN.

This electrical activity started immediately before the respiratory reflex and continued as long as the respiration was augmented, and gradually vanished with the subsidence of the respiratory reflex.

In order to get better records of these small potentials, the nerve was split up into tiny filaments. A record from such a filament, in which the number of the larger pressure potentials elicited by each pulse wave is greatly reduced, is seen in fig. 1 C, and the effects of cyanide and lobeline are seen below (fig. 1 D and E).

From these records it is evident that cyanide and lobeline produce the same type of change in the electric response, i. e. a massive discharge of small action potentials, which is consistant with our experiences with the cat. The records further show that neither lobeline nor cyanide give rise to any change in the discharge of the larger action potentials occurring at each pulse wave.

In a previous report (EULER, LILJESTRAND and ZOTTERMAN 1941 b) it was shown that the cat's sinus nerve contains thin fibres, which respond to variations in the intrasinal pressure, but are insensitive to chemical stimuli, such as oxygen want, carbon dioxide, and synaptotropic substances, such as, for example, lobeline in non-paralyzing concentrations. It seemed therefore of interest to investigate whether, in the dog, there exist any pressure fibres giving rise to potentials of the same small size as those produced by lobeline and cyanide. For that purpose we have made perfusion experiments on the sinus. A cannula was inserted in the common carotid artery, and all arteries except the occipital artery were tied above the sinus region. The sinus was perfused with oxygenated heparinized dog's blood. In this experiment the nerve preparation happened to be quite free from any of the larger pressure potentials. At a perfusion pressure of 100 mm Hg there appeared only a fairly small number of small irregularily shaped action potentials (fig. 2 A). As will be seen from fig. 2 C and E, a rise in pressure produced a regular increase in this electrical activity, thus demonstrating that the dog's sinus nerve contains a large number of baroceptive fibres of small size. As will be seen from fig. 2 B, an injection of 3 ml of a Ringer solution containing 10 μg lobeline in the rubber tubing near the cannula produced a very massive response of small action potentials. Such a response, which is consistent with the effect produced by an intravenous injection of lobeline, seen above from fig. 1 B, could also be produced by cyanide (see fig. 2 D). An injection of 10 μg of acetylcholine elicited a short volley of small potentials in conformity with

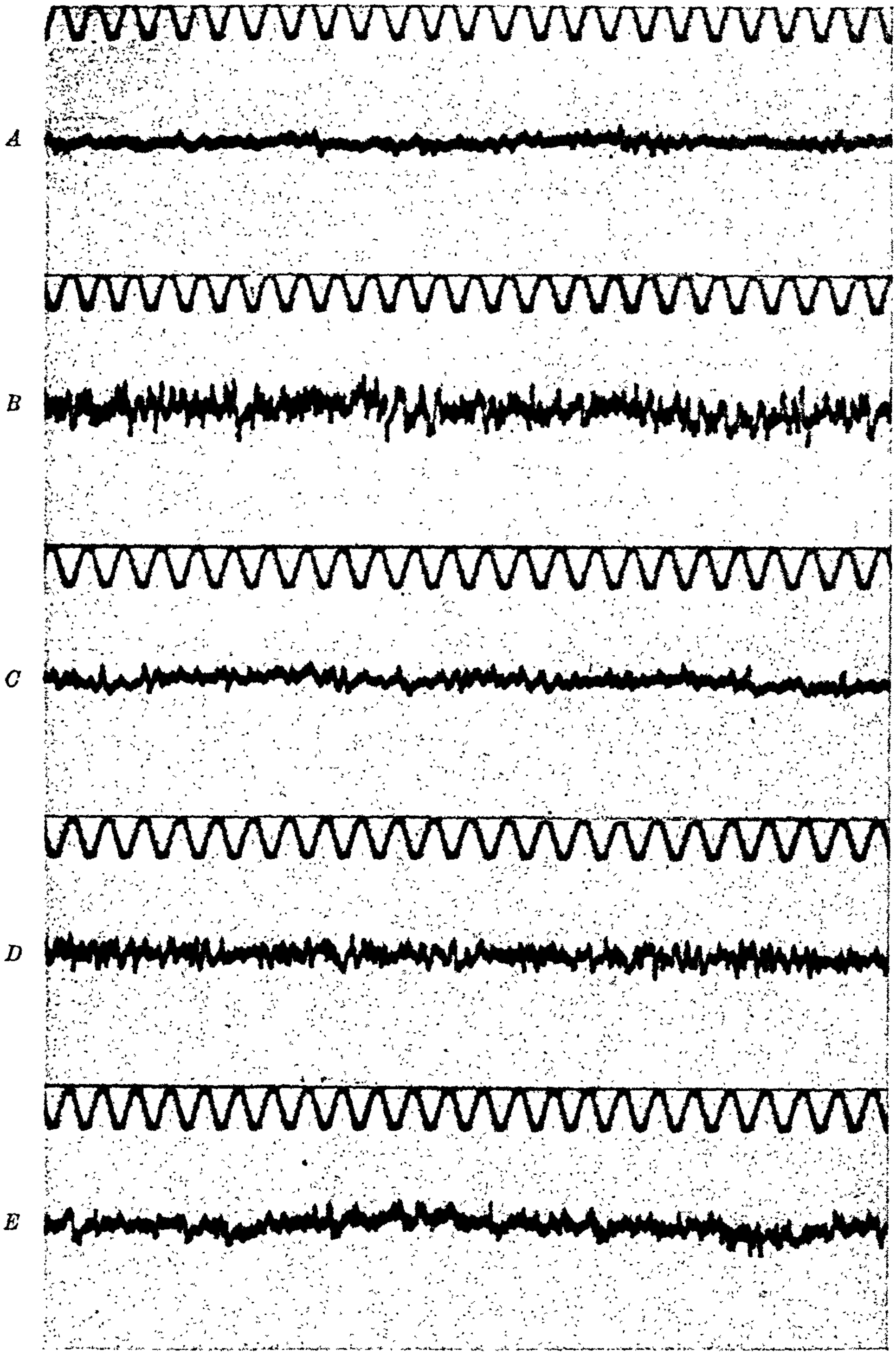


Fig. 2. Dog, chloralose. Action potentials from the sinus nerve while perfusing the left isolated carotid sinus with oxygenated heparinized blood. In the preparation no pressure potentials of large sizes. *A*. Perfusion pressure 100 mm Hg. *B*. After an injection of 3 ml of a Ringer solution containing 10 μg of lobeline into the perfusion cannula; perfusion pressure 100 mm Hg. *C*. and *D*. blood pressure 120 mm Hg. *D*. after injection of 3 ml of a weak KCN solution. *E*. Perfusion pressure 180 mm.

our experience with the cat (EULER, LILJESTRAND and ZOTTERMAN 1941 a). A control injection of 3 cc Ringer solution had no visible effect.

These findings naturally raise the question whether the pressure and the applied chemical stimuli act on the same kind of fibres, or whether each kind of stimulus affects their specific fibres. This problem can hardly be solved for the dog by studying the activity of single fibres, since their isolated axone potentials are too small. We have also failed in our attempts to exclude all the baroceptive fibres by ligating the sinus just below the place where the occipital artery branches off. There are, nevertheless, a number of facts which not only support the view that the small fibres in the sinus nerve are of two functionally specific kinds, but also definitely speak against the possibility of identical fibres.

1) Lobeline and cyanide in weak doses greatly increase respiration reflexly from the carotid body, whereas a rise in the intrasinusal pressure rather diminishes the respiratory activity.

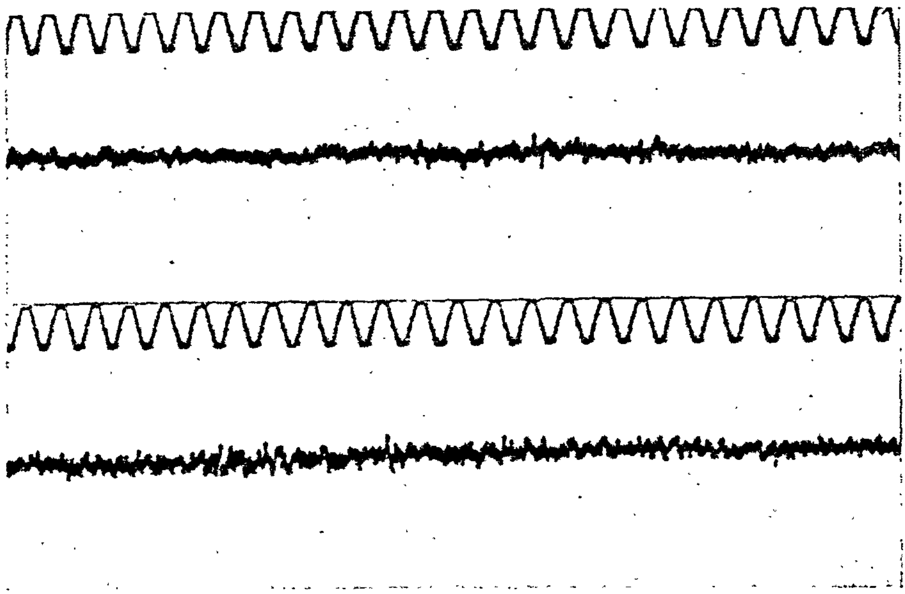
2) Lobeline and cyanide increase the systemic blood pressure reflexly from the carotid body, while it is lowered by an increase of the intrasinusal pressure.

3) BOUCKAERT, DAUTREBANDE and HEYMANS (1931) and later COMROE and SCHMIDT (1938) have shown that the baroreceptors in the dog do not respond to chemical stimuli. Further, a study of the action potentials (EULER, LILJESTRAND and ZOTTERMAN 1941 b) has shown that the small baroceptive fibres in the cat are stimulated neither by anoxemia, by carbon dioxide nor by lobeline and other synaptotropic substances.

All these facts seem to us to warrant the view that the small action potentials in the dog's sinus nerve derive from two different kinds of fibres, one kind specifically reacting to stretch, and the other kind to chemical stimuli.

In order to test the effect of anoxemia on the perfused sinus, the blood was exchanged for deoxygenated Ringer solution at the same pressure. This brought about a small, but quite significant increase in the electrical activity (fig. 3), which is in accordance with our observations on the cat, that even a high degree of hypoxemia never gives such a strong electrical response from the chemical fibres as does lobeline.

Unfortunately the chemical potentials in the dog are too small in our records to allow of a quantitative analysis. Our experiments however, have not provided any facts to show that the dog's



A

B

Fig. 3. Records from the same preparation as in fig. 2. *A*. Perfusion with oxygenated blood, pressure 120 mm Hg. *B*. Perfusion with deoxygenated Ringer solution at the same pressure.

chemoreceptors principally react in any other way than we have found to be the case with the cat, for which animal we have presented quantitative data (cf SCHMIDT and COMROE 1941). These authors criticise the view expressed by EULER and LILJESTRAND (1936), HEYMANS and BOUCKAERT (1938), and EULER, LILJESTRAND and ZOTTERMAN (1939), that carbon dioxide, under physiological conditions, reflexly leads to an influence on the ventilation. We based our opinion upon a quantitative study of the relation between the carotid chemoreceptive activity and the alveolar carbon dioxide in the cat. As our experiments, in agreement with SAMAAN's and STELLA's (1935) observations, definitely showed that the chemoreceptors start discharging at an alveolar carbon dioxide tension just below 30 mm Hg, and that the intensity of the response increases rather in direct proportion to the alveolar carbon dioxide, we consider it fully justifiable to assume that the chemoreceptors of the carotid region cooperate in the physiological regulation of respiration by carbon dioxide in the cat. This view has been further corroborated by the fact that denervation of both sinuses leads to a decrease in the spontaneous ventilation accompanied by an increase in the alveolar carbon dioxide, even when the animal is breathing oxygen instead of air (EULER and LILJESTRAND 1940).

Summary.

1. The electroneurogram of the dog's carotid sinus nerve reveals a train of potentials of various sizes forming compact volleys at each systole. All the larger and medium-sized potentials derive from *baroceptive fibres*.

2. Apart from the larger potentials, there appears an electrical activity built up by axone potentials of very small spike-heights in response *to* both chemical *stimuli and intrasinusal pressure*.

3. Evidence is presented to show that these small axone potentials derive from two functionally different kinds of fibres, one kind specifically reacting to intrasinusal pressure, the other kind responding to chemical stimuli.

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Über die reflektorische Beeinflussung der Atmung durch den endosinualen und endoaortalen Druck.

Von

H. BJURSTEDT und U. S. v. EULER.

(Eingereicht am 3 April 1942.)

Neuerdings haben KRAMER und GAUER (1941) in einer Untersuchung über die Arbeitshyperpnoe diese zum Teil auf eine Entlastung der Barorezeptoren im Carotissinusgebiet zurückgeführt. Eine Voraussetzung dieser Betrachtungsweise ist allerdings, dass die Atmung normalerweise durch Verminderung des endosinualen Druckes tatsächlich gesteigert wird, und zwar reflektorisch über die drucksensiblen Rezeptoren, d. h. dass die Atmung tonisch gehemmt ist. Trotz zahlreicher Untersuchungen über dieses Thema scheint die Frage noch nicht endgültig geklärt zu sein.

Atmungsbemmung als Folge einer Druckerhöhung im Sinusgebiet, und umgekehrt Verstärkung der Atmung nach Drucksenkung, wie z. B. bei Carotidenabklemmung, ist von vielen Untersuchern beschrieben worden (MOISSEJEFF, 1926—27, HEYMANS 1928 a, HERING 1929, HEYMANS und BOUCKAERT, 1930, KOCH und MARK, 1931, GOLLWITZER-MEIER und SCHULTE, 1931—32, SCHMIDT, 1932, HEYMANS, BOUCKAERT und DAUTREBANDE, 1932).

Durch die Entdeckung der Chemorezeptoren durch HEYMANS, BOUCKAERT und DAUTREBANDE (1930) wurde die Frage nach dem Auslösungsmechanismus der respiratorischen Reflexe komplizierter, da eine Drucksenkung im Sinusgebiet nicht nur eine Entlastung der Barorezeptoren mitführt, sondern auch die Durchblutungsverhältnisse im Glomus verändert und zwar in der Rich-

tung einer relativen Hypoxie. Die überaus grosse Empfindlichkeit des Glomus für hypoxische Erregung (EULER, LILJESTRAND und ZOTTERMAN, 1939) macht es notwendig, der letzterwähnten Möglichkeit besondere Aufmerksamkeit zu widmen (GAYET, BENNATI und QUIVY, 1935, SAMAAN und STELLA, 1935, EULER und LILJESTRAND, 1936, 1937). Aus der Literatur geht ferner hervor, dass die atmungshemmende Wirkung der Druckerhöhung im Sinusgebiet bisweilen unbedeutend ist oder fehlt (KOCH, 1929, GOLLWITZER-MEIER und SCHULTE, 1931—32, SCHMIDT, 1932), wozu jedoch bemerkt werden soll, dass die hierbei reflektorisch eintretende, allgemeine Blutdrucksenkung über die Vago-Depressoren antagonistisch wirken kann. In gewissen Fällen kann sogar eine Atmungssteigerung als Folge einer Sinusdruckerhöhung erfolgen (SCHWEITZER, 1934—35, GUPTA, 1936, WINDER, 1937, EULER und LILJESTRAND, 1937). Offenbar beteiligen sich dabei verschiedenartig wirkende Faktoren. Als Ursache für die »paradoxe» Atmungssteigerung bei Carotidendruckerhöhung haben die letzterwähnten Autoren (l. c. Fig. 7) eine Erregung unspezifischer, sensibler Nervenfasern im Carotissinusgebiet angenommen, da Reizung sensibler Nerven in anderen Gebieten einen ähnlichen Effekt hervorruft. Die Auffassung, dass die Atmungsverminderung bei Sinusdruckerhöhung auf einer Entlastung der Chemorezeptoren beruht, wurde dadurch gestützt, dass bei Perfusion des Sinusgebiets mit sauerstoffarmem Blut eine Atmungshemmung bei Druckerhöhung nicht mehr zum Vorschein kam. Die Atmung blieb hierbei hochgradig gesteigert (l. c. S. 200. Siehe auch MARRI und HAUSS, 1939). In einer sorgfältigen Untersuchung hat WINDER (1938) die Frage bezüglich des pressorezeptorischen Atmungsreflexes näher studiert, unter Berücksichtigung sowohl chemischer Reflexe als durch den Sinusdruck verursachter Änderungen des systemischen Blutdruckes. Er suchte die Chemorezeptoren durch Embolisierung auszuschalten und benutzte ferner eine kompensatorische Vorrichtung, um den allgemeinen Blutdruck konstant zu halten. Ausserdem wurde die Ventilation konstant erhalten und die Atembewegungen quantitativ registriert. Er kam dabei zu dem Schlusse, dass ein pressorischer Atmungsreflex (Dehnungsreflex) tatsächlich vorkommt. Leider werden keine Beweise dafür erbracht, dass die zirkulatorische Ausschaltung der Chemorezeptoren vollständig war (z. B. durch Prüfung mit Cyanid), oder dass die Atmungshemmung nach Inaktivierung der Pressorezeptoren restlos verschwand. Jedenfalls ist zu verzeichnen, dass in WIN-

DERS Versuchen die Erhöhung des endosinualen Druckes von 0 auf 255 mm Hg eine anfangs recht bescheidene Atmungshemmung herbeiführte, die zwar später verstärkt wurde (am vagotomierten Tier). Wenn es sich um eine pressoreflektorische Hemmung handelte, sollte man eher erwarten, dass die Wirkung anfangs am stärksten war. Auffallend ist ferner, dass nach Senkung des endosinualen Druckes auf 0 die Atmung erst allmählich auf ihren vorigen Umfang zurückging. Man kann sich schwerlich des Verdachts wehren, dass die Zirkulation im Glomusgebiet nicht vollständig aufgehoben war, und im besonderen dass die steile Druckerhöhung auf 255 mm Hg neue Blutwege geschaffen hat (bei gleichzeitiger reflektorischer Vasodilatation), wodurch die wahrscheinlich maximal hypoxische oder asphyktische Erregung der Chemorezeptoren allmählich vermindert wurde.

Mit einer abweichenden Technik haben im 1939 MARRI und HAUSS in Heymans' Institut die Frage erneut angegriffen. Sie haben einen umgestülpten Venensack (LIM und CHANG, 1936) im Sinus nach dem Verfahren von HEYMANS, DONATELLI und SHEN (1938) eingebracht und den Sinusdruck mittels Inflation, bzw. Deflation des Venensackes verändern können. Dabei wurde an vagotomierten Tieren sehr ausgesprochene Atmungshemmung bei Druckerhöhung registriert, die zweifelsohne auf eine reflektorische Erregung der Barorezeptoren zurückzuführen ist.

Die Mehrzahl der Versuche, wo eine deutliche Atmungshemmung durch erhöhten Sinusdruck hervorgerufen ist, sind an vagotomierten Tieren gemacht. Bei erhaltenen Vago-Depressoren bleibt die Wirkung oft aus oder ist umgekehrt. Als Ursache dieser Verschiedenheit wird meistens angenommen, dass eine antagonistische Wirkung über die Vago-Depressoren stattfindet, die von der Blutdrucksenkung ausgelöst wird.

In letzterer Zeit haben BJURSTEDT und HESSER (1942) die Einwirkung des Sinusdruckes auf die Atmung der Katze studiert unter Benutzung voll arterialisierten Heparinblutes als Perfusionsflüssigkeit. Sie konnten dabei keine sichere Wirkung von Sinusdruckveränderungen auf die Atmung bei erhaltenen Vago-Depressoren feststellen. Andererseits sprach die Atmung auf chemische Erregung prompt an. Nach Ausschaltung der Vago-Depressoren trat dagegen eine deutliche Hemmung der Atmung bei Erhöhung des Perfusionsdruckes ein.

In Anbetracht der etwas unklaren Ergebnisse an Hunden und des Fehlens streng beweisender Versuche an Tieren mit erhaltenen

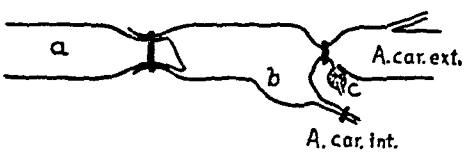
Vagodepressoren schien es uns von Intresse, die Frage nochmals einer Prüfung mit teilweise neuer Methodik zu unterwerfen.

Methodisches.

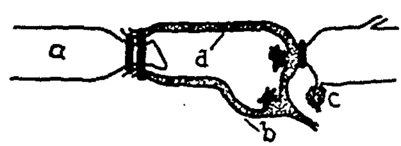
Die Versuchstiere waren Hunde von 7—15 kg Gewicht, die mit 10 ml 1 % Chloralose pro kg Körpergewicht narkotisiert waren; in einigen Fällen mit Zugabe von 1 mg Opiototal Astra (50 % Mo^{+}) pro kg. Die Reflexerregbarkeit der Atmung und des Kreislaufs waren in dieser Narkose gut erhalten.

Die Atmung wurde durch Einschliessen der Tiere in einem Kasten pletysmographisch quantitativ registriert. Der Blutdruck wurde in der A. femoralis mittels Hg-manometer gemessen.

Um Wirkungen seitens Variationen im allgemeinen Blutdruck zu vermeiden, wurde in einigen Versuchen ein Blutdruckskompensator



1 a.



1 b.

Fig. 1 a. Schematische Darst llung der Versuchsanordnung bei Perfusion des isolierten Barorezeptorgebietes. a Kanüle in der A. carotis communis, b Sinus, c A. occipitalis mit dem Glomus caroticum. 1 b. Umgestülpter Venensack (d) im Sinus.

in das arterielle System eingeschaltet, der aus einer Druckflasche mit arterialisertem Heparinvollblut bestand, die mit der Arteria femoralis verbunden war und mittels eines Quecksilberventiles und Sauerstoffdruckzylinders auf den gewünschten Druck eingestellt werden konnte. Es wurde frisches Rinderblut verwendet, dessen Kompatibilität mit dem Blut des Tieres geprüft wurde. Die Druckflasche mit dem Blut wurde während des Versuches auf 38° gehalten. Die Verbindungsschläuche waren kurz und genügend weit, um merkliche Druckgefälle zu verhindern. Nahe der Femoraliskanüle wurde ein Seitenast auf der Leitung nach der Druckflasche an den Hg-manometer angeschlossen. Das Auftreten von Pulsationen am Manometerdruck sowie das anfänglichen Beibehalten des Blutdruckes des Tieres nach Abklemmung der Leitung zur Druckflasche zeigte, dass der Druckausgleich schnell und vollständig erfolgte.

Die Druckvariationen im Sinusgebiet wurden mittels einer in die Carotis communis eingebundenen Kanüle, die ebenfalls mit einer mit Blut beschickten Druckflasche verbunden war, bewerkstelligt. Da eine Wirkung nur auf die Barorezeptoren beabsichtigt wurde, wurde die A. carotis externa unmittelbar oberhalb der Bifurkation abgebunden, d. h. vor dem Abgang der den Glomus versorgenden A. occipitalis. (Comroe und Schmidt, 1938) (Fig. 1). Die reflektorische Ansprechbarkeit der Barorezeptoren und der Chemorezeptoren konnte zu jedem

beliebigen Zeitpunkt mittels endosinualen Druckvariationen, bzw. Atmung einer sauerstoffarmen Gasmischung geprüft werden. Die letztere erfolgte nach Einschalten von Müllerventilen.

In einem Versuch wurde ein Venensack nach HEYMANS, DONATELLI und SHEN, benutzt, um Druckvariationen im Sinus herbeizuführen.

Ergebnisse.

a) *Wirkung von Änderungen des allgemeinen Blutdruckes bei Erhaltung sämtlicher Druck- und Chemorezeptoren.*

Die Wirkung von Erregung oder Entlastung der Druckrezeptoren auf die Atmung sollte bei allgemeiner Blutdruckerhöhung oder -Senkung studiert werden können, wobei die Druckänderungen durch Ein- und Auslauf von Blut herbeigeführt werden. Eine Voraussetzung hierfür ist zwar, dass die Chemorezeptoren nicht in wechselndem Umfange miterregt werden. Dass bei Luftatmung eine gewisse »Ruheerregung» der Chemorezeptoren stattfindet, ist früher u. a. von SELLADURAI und WRIGHT (1932—33), EULER und LILJESTRAND (1936, 1940, 1942), SAMAAN und STELLA (1935) sowie von EULER, LILJESTRAND und ZOTTERMAN (1939) wahrscheinlich gemacht worden, sowohl bezüglich der Wirkung von Hypoxie wie von Kohlensäure. Eine reflektorische Veränderung der Blutdruckströmung durch den Glomus lässt sich zwar in den hier geschilderten Versuchen nicht vermeiden, da eine allgemeine Drucksteigerung eine Vasodilatation herbeiführt und umgekehrt Drucksenkung Vasokonstriktion verursacht. Etwaige Rückwirkungen auf die Chemorezeptoren aus diesem Grund konnten aber durch Sauerstoffatmung gewissermassen neutralisiert werden. Nur bei niedrigen Drucken besteht dann die Gefahr einer hypoxischen Erregung der Chemorezeptoren.

Bei allgemeiner Druckerhöhung trat regelmässig eine gewisse Verminderung der Atmung ein, (Fig. 2 A) die aber bei Sauerstoffatmung nur in unbedeutendem Grad verändert wurde. Die Atmungshemmung kann deshalb kaum auf eine verbesserte Durchblutung des Glomus zurückgeführt werden. Da aber die allgemeine Druckerhöhung nebst reflektorischer Vasodilatation auch eine Vermehrung der Blutfüllung der Lungen herbeiführt, darf es nicht übersehen werden, dass hierdurch ein verstärkter Hering-Breuer-Reflex erfolgen kann. Gerade die in der Fig. 2 A zu beobachtende Hemmung der Inspirationsbewegungen bei zunehmender Blutfüllung des Tieres ist mit einer solchen Deutung gut vereinbar.

Es kann somit die bei allgemeiner Druckerhöhung beobachtete Atmungsverminderung, die ausserdem recht gering ist, nicht mit Sicherkeit auf eine pressoreflektorische Wirkung in den Aorta- und Sinusgebieten zurückgeführt werden.

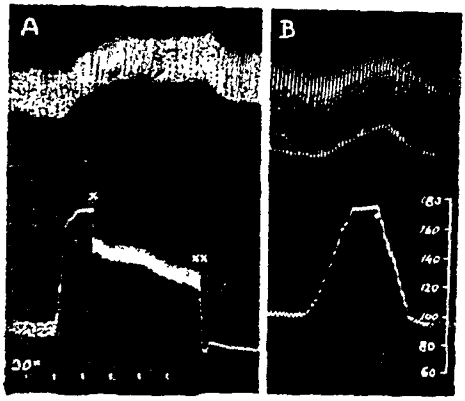


Fig. 2. Hunde, Chloralose. Obere Kurve Atmung, untere Kurve allgemeiner Blutdruck. A. Drucksteigerung durch Blutinfusion, beendet bei x. Bei xx Drucksenkung durch Blutausströmung. B. nach Durchtrennung der Vagi-Depressoren. Drucksteigerung und -Senkung durch Ein- und Auslauf von Blut.

b) *Wirkung von Änderungen des Sinusdruckes bei erhaltenen Baro- und Chemorezeptoren unter kontrolliertem allgemeinem Blutdruck.*

Versuche dieser Art scheinen uns am besten geeignet, der Frage nach den Wirkungen von Erregung der Barorezeptoren des Sinusgebietes auf die Atmung näher zu kommen. Hierbei werden erstens die für die Atmung wichtigen Vagusnerven erhalten, wie stets unter normalen Bedingungen, und ferner werden etwaige Nebenwirkungen auf Grund von sekundären Blutdruckvariationen vermieden. Die Versuche haben eindeutig gezeigt, dass auch in Tieren, wo die Reflexerregbarkeit sehr hoch ist, Sinusdruckvariationen ohne wesentlichen Einfluss auf die Atmung sind (Fig. 3 A). Von einer Hemmung, wie sie in den unten zu beschreibenden Versuchen unter anderen Versuchsbedingungen beobachtet wird, ist nichts zu sehen; man beobachtet vielmehr meist eine leichte Steigerung, wie sie früher in den Versuchen von Euler und Liljestrand (1937) und Winder (1937) an nicht vagotomierten Tieren gefunden worden ist. Aus diesen Versuchen geht hervor, dass eine Antagonistwirkung des Aortagebietes — oder anderer reflexogener Gebiete — bei allgemeiner Blutdrucksenkung die abweichende Ergebnisse bei erhaltenen Vagi nicht erklären kann.

c) *Wirkung von Änderungen des allgemeinen Blutdruckes nach Ausschaltung der Vago-Depressoren.*

Wenn die unter a) erwähnten Versuche nach Durchschneidung der Vago-Depressoren (nebst Halssympathicus) wiederholt wurden, war die Wirkung von systemischen Blutdrucksvariationen

auf die Atmung kleiner und manchmal unsicher (Fig. 2 B). Wenn jedoch der allgemeine Blutdruck auf niedrige Werte (80 mm Hg oder niedriger) gesenkt wurde, trat eine Atmungssteigerung ein, wahrscheinlich als Folge einer hypoxischen Erregung des Glomus caroticum. Auch zeigte sich bisweilen ein besonders auffallender Wirkungsunterschied von Druckvariationen bei Luft- oder Sauerstoffatmung (Fig. 4, A, B). Dies lässt sich, wie oben angeführt, dadurch erklären, dass die Sauerstoffversorgung des Glomus bei Luftatmung des narkotisierten Tieres von dem Durchblutungsdruck weitgehend beeinflusst wird. Bei Sauerstoffatmung kann man demgemäss den Blutdruck auf ein niedrigeres Niveau senken, bevor eine hypoxische Erregung des Glomus eintritt.

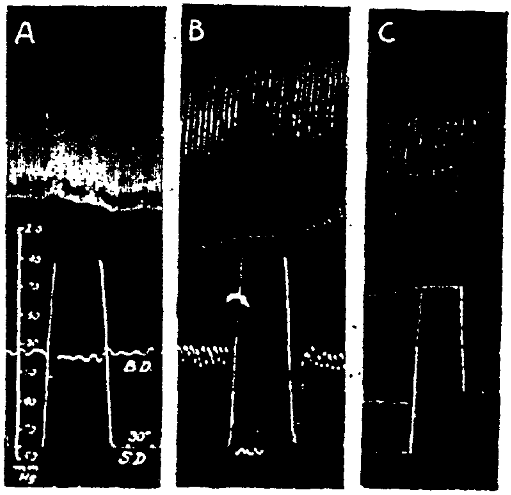


Fig. 3. Hunde, Chloralose. Obere Kurve Atmung. B. D. allgemeiner Blutdruck, S. D. endosinualer Druck. A. Vago-Depressoren erhalten, kontrollierter Blutdruck. B. Vago-Depressoren durchschnitten, nicht kontrollierter Blutdruck. C. Wie B aber kontrollierter Blutdruck.

Bei dieser Versuchsanordnung fällt bei allgemeiner Drucksteigerung durch Bluteinlauf sowohl die durch Hemmung des Vasokonstriktorentonus verursachte reflektorische Erweiterung der Gefässe des Glomusgebietes als auch der Hering-Breuer'sche Reflex aus. Es dürfte berechtigt sein, den Wegfall dieser Momente in die Erklärung der verminderten Wirkung einzubeziehen.

d) Wirkung von Änderungen des endosinualen Druckes nach Ausschaltung der Vago-Depressoren.

Bei diesen Versuchen wurde wie unter b) nur ein Sinus Druckänderungen ausgesetzt, während der zweite denerviert war.

In diesen Versuchen wurde in Bestätigung der Befunde früherer Untersucher eine Senkung der Atmungsfrequenz regelmässig beobachtet, wobei die Amplitude entweder vermindert, erhöht oder unverändert blieb. Die Frequenzminderung war in der Mehrzahl der Fälle mässig (Fig. 3 B und C), es wurden aber auch

sehr ausgesprochene Wirkungen beobachtet (Fig. 5). Ob hierbei der allgemeine Blutdruck konstant gehalten wurde oder nicht, schien für den Effekt wenig von Belang zu sein. Vollkommen ähnlich verliefen die Versuche bei Verwendung eines umgestülpten Venensackes nach HEYMANS, DONATELLI und SHEN.

Ein beeinträchtigender Faktor wäre hier nur die durch passive Mehrdurchblutung (nach Durchschneidung des Halssympathicus)

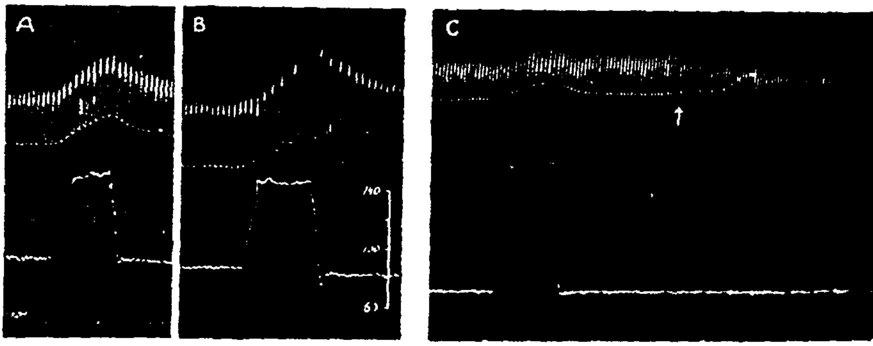


Fig. 4. Hunde, Chloralose. Obere Kurve Atmung, untere Kurve allgemeiner Blutdruck, durch Ein- und Auslauf von Blut kontrolliert. Vago-Depressoren durchschnitten. A. Sauerstoffatmung. B. Luftatmung. C. nach Entnervung beider Sinusgebiete. Bei ↑ Einatmung von 5,2 % O_2 in Stickstoff statt Sauerstoff.

verursachte Veränderung der Durchströmungsverhältnisse im Glomusgebiet, die deshalb besonders beeinflussbar wären, weil die Durchblutung aus dem Externagebiet geregelt wird, das selbst retrograd versorgt wird. Da O_2-Atmung am Atmungseffekt der Drucksteigerung kaum etwas änderte, glauben wir indessen dass der Effekt reflektorisch über die Barorezeptoren zustande kommt.

e) *Wirkung von Änderungen des systemischen Blutdruckes bei ausgeschalteten Baro- und Chemorezeptoren.*

Die oft geringfügigen Atmungsreaktionen bei Variationen im allgemeinen Blutdruck nach Durchtrennung der Vagodepressoren (c) können ja nur dann als spezifisch bedingt angesehen werden, wenn die Wirkung nach Ausschaltung der reflexogenen Gebiete verschwindet. Dies war aber nicht der Fall, obschon die Vollständigkeit der Denervation eingehend geprüft wurde (Fig. 4 C). Nach der Ausschaltung der reflexogenen Aorta- und Sinusgebiete war die Atmung in der Regel vermindert, wie EULER und LILJESTRAND (1940) gezeigt haben. Erhöhung des allgemeinen Blut-

druckes führte auch jetzt eine leichte Verminderung der Atmung herbei, was möglicherweise auf einer Erhöhung des intrakranialen Druckes beruht (HEYMANS, 1928 b).

Besprechung.

Es steht ausser Zweifel, dass in vielen Fällen, wo eine lokale Druckänderung im Sinusgebiet eine Veränderung der Atmung hervorgerufen hat, diese zum Teil auf die Durchblutungsverhältnisse im Glomus zurückzuführen ist, was ja mit Kenntnis der hohen Empfindlichkeit der Chemorezeptoren für die Sauerstoffversorgung nicht überraschend ist. Anderseits scheint die von mehreren Autoren beobachtete Hemmung der Atmung bei Sinusdruckerhöhung — besonders in den Versuchen von MARRI und HAUSS — auf eine reflektorische Wirkung über die Barozeptoren zu beruhen. Die uns zunächst interessierende Frage war die, ob unter normalen Bedingungen, d. h. bei erhaltenen Vagi, diese Wirkung sich geltend machen kann? Wir haben indessen keine Zeichen dafür finden können; im Gegenteil fanden wir in Bestätigung von EULER und LILJESTRAND (1937) und WINDER (1937) oft eine Steigerung der Atmung bei Sinusdruckerhöhung, ohne dass eine Antagonistwirkung anderer reflexogener Zonen in unseren Versuchen mitspielen konnte. Wie aus den vorliegenden Versuchen hervorgeht, scheint die Ursache der widersprechenden Resultate darin zu liegen, dass in denjenigen Versuchen, wo die Sinusdruckhemmung einwandfrei demonstriert worden ist, immer die Vago-Depressoren durchtrennt waren. Wenn in ein und demselben Versuch der Sinusdruck einerseits bei erhaltenen und andererseits bei durchschnittenen Vago-Depressoren erhöht wird, findet man im ersten Falle keine Wirkung oder eine leichte Frequenzsteigerung (Fig. 3) und im zweiten Falle regelmässig eine Hemmung (Fig 3 und 5), sowohl bei frei gelassenem oder konstant gehaltenem allgemeinem Blutdruck. Unsere Fragestellung kann deshalb dahin beantwortet werden, dass zwar eine atmungshem-

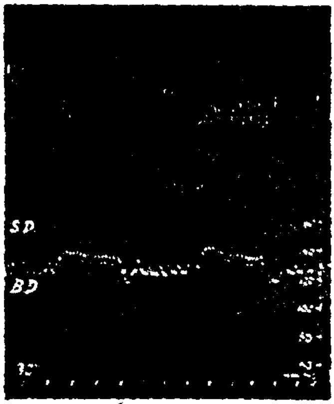


Fig. 5. Hunde, Chloralose. Obere Kurve Atmung, S. D. endosinualer Druck, B. D. allgemeiner Blutdruck, durch Kompensatorvorrichtung konstant gehalten.

mende Wirkung von endosinualer Druckerhöhung sich unter besonderen Umständen (Ausschaltung der Vago-Depressoren) nachweisen lässt, dass aber normalerweise keine solche Wirkung die Atmung merklich beeinflusst. Hieraus folgt, dass unter physiologischen Bedingungen mit einer Atmungsverstärkurg auf Grund einer Enthemmung der Barorezeptoren (KRAMER und GAUER) nicht zu rechnen ist.

Zusammenfassung.

1. Allgemeine Blutdrucksteigerung durch Blutinfusion verminderte die Atmungsamplitude bei unveränderter oder erhöhter Frequenz am chloralosierten Hund (Inspirationshemmung).

2. Nach Durchtrennung der Vago-Depressoren wurde diese Wirkung vermisst. Bei mittleren Ausgangsdrucken und Sauerstoffatmung trat eine mässige Frequenzminderung bei Drucksteigerung zutage, die bei Luftatmung oder bei niedrigen Ausgangsdrucken, wo die Atmung von vornherein erhöht war, verstärkt wurde (Entlastung der Chemorezeptoren).

3. Lokale endosinuale Druckerhöhung rief bei erhaltenen Vago-Depressoren keine Atmungshemmung hervor, auch nicht bei konstant gehaltenem Blutdruck. Meist wurde eine leichte Frequenzzunahme beobachtet.

4. Lokale endosinuale Druckerhöhung nach Durchtrennung der Vago-Depressoren rief regelmässig eine Frequenzminderung hervor, auch bei konstant gehaltenem Blutdruck.

5. Die Versuchsergebnisse haben keine Anhaltspunkte dafür gegeben, dass unter normalen Bedingungen die Atmung von den Barorezeptoren im Aorta- und Sinusgebiet reflektorisch beeinflusst wird, wie dies nach Durchtrennung der Vago-Depressoren der Fall ist.

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Influence of Oxygen Inhalation on the Chemoreceptor Activity of the Sinus Region.

By

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During spontaneous breathing of air, anaesthetized animals often develop a certain degree of desaturation of the arterial blood. Thus cats anaesthetized with 0.06 g chloralose per kg intravenously, which produces an adequate anesthesia for experimental operative purposes, showed a saturation of the arterial blood of about 90 per cent. This moderate desaturation was sufficient to act as a stimulus for the highly sensitive chemoreceptors of the carotid sinus region, as shown by the "chemical" impulses in the nerve of Hering (EULER, LILJESTRAND and ZOTTERMAN 1939). In fact, nerve impulses deriving from the chemoreceptors as a result of oxygen want could be recorded even when the saturation of the hemoglobin exceeded 90 per cent.[1] The local origin of the impulses was proved by the fact, among others, that the typical action potentials from the sinus nerve were increased, not only when a certain degree of desaturation occurred in the circulating blood, but also when a purely local asphyxial stimulation was produced by clamping the common carotid artery. As stated in our paper, it was only possible to keep the response from the nerve at a low frequency under these conditions when the cat was

[1] The degree of saturation of the arterial blood is an indicator of the arterial oxygen pressure, but neither the former nor the latter, as ASMUSSEN and CHIODI (1941) claim, would, as such, be the determining factor for the oxygen supply in the glomus. Both may, however, conveniently be used as a relative measure when the circulatory rate, the oxygen consumption in the reacting tissues, and the oxygen capacity of the blood are unchanged.

considerably overventilated with oxygen. SCHMIDT and COMROE (1940), being unable to confirm these findings in the dog, have expressed some doubts as to the interpretation of the results obtained by EULER, LILJESTRAND and ZOTTERMAN. In a recent paper EULER and ZOTTERMAN (1942) have critisized their arguments and shown that similar results can be obtained in the dog with a suitable technique.

These results are in full accord with our finding (EULER and LILJESTRAND 1940) that the inhalation of oxygen in the anesthetized cat, as well as in the dog, caused a temporary decrease in ventilation, indicating that a stimulus from oxygen want is removed. If the sinus region had been denervated, oxygen inhalation gave no decrease of ventilation. DUMKE, SCHMIDT and CHIODI (1941) also reported in the dog that, in some cases, inhalation of oxygen caused transitory respiratory depression, while the chemoreceptors were active; after chemoreceptor denervation of the sinuses, oxygen inhalation regularly caused stimulation of respiration. RUDBERG (1941) found that the stimulating effect on respiration of clamping the carotid arteries in cases with low arterial pressure was greatly reduced or abolished by oxygen inhalation. Reduction of the perfusion pressure in the circulatory isolated sinus region also stimulated respiration, as a result of relative oxygen lack (EULER and LILJESTRAND 1937). The experiments quoted have proved that a stimulating effect on respiration is brought about by a relatively small degree of desaturation of the hemoglobin. This paper adduces some fresh evidence on this point.

It is well known that the stimulation of the chemoreceptors in the sinus region may influence, not only respiration, but also circulation. (HEYMANS, BOUCKAERT, EULER and DAUTREBANDE 1932, EULER and LILJESTRAND 1936, WINDER, BERNTHAL and WEEKS 1938, BOUCKAERT, GRIMSON, HEYMANS and SAMAAN 1941). Such effects have been observed during considerable hypoxia or hypercapnia, but very little is known about their rôle, when the animals under experimentation have been breathing air, thus under approximately physiological conditions. In view of the high sensitivity of the chemoreceptors for variations in the chemical composition of the blood and the reflex actions observed on respiration, it seemed of interest to study whether corresponding effects on blood pressure and heart rate could be demonstrated.

Methods.

Cats and dogs were used as experimental animals and anesthetized with 0.06 and 0.1 g chloralose per kg body-weight intravenously. In order to reduce shivering in the dogs, which was sometimes rather pronounced, barbiturates were mostly given in additional small doses.

Respiration was measured quantitatively by means of the body plethysmograph described elsewhere (EULER and LILJESTRAND 1936). Arterial blood pressure was recorded with a Hg-manometer from the femoral artery. The pulse rate was measured for a period of 20—30 seconds.

In most cases the animals were breathing spontaneously through Müller valves, permitting the inhalation of various gas mixtures contained in rubber bags. In order to avoid the initial dilution of the oxygen when changing from air to oxygen, a second set of Müller valves, previously filled with oxygen, was sometimes used. In this way an instantaneous reversal from air to oxygen (or in the opposite direction) could be obtained. Sometimes the animals were artificially ventilated by a respiration pump, to which gas bags were attached when gas mixtures other than air were used. Over-ventilation was carefully avoided, and the artificial ventilation was maintained at a constant level throughout the experiment.

Denervation of the sinus region was accomplished by tying off the tissues between the internal and external carotids about 5 mm above the bifurcation or by section of the glossopharyngeal nerves centrally to the entrance of the sinus nerves. The completeness of the denervation was proved by the disappearance of the stimulating effect of 7 per cent oxygen in nitrogen on respiration.

Results.

I. Respiration.

In all experiments where the influence of spontaneous breathing of oxygen was studied, a sufficiently long period of air breathing was previously maintained at a constant rate and with constant ventilation. When oxygen was given, we regularly obtained a decrease in ventilation, as described earlier (EULER and LILJESTRAND 1940). This reaction was observed in anesthetized cats and dogs, and also in decerebrate cats, and consisted in a reduction of respiratory frequency and of amplitude as well. The reduction in ventilation was often very marked, though generally it did not last long, and after some minutes the ventilation was only moderately reduced (cf. EULER and LILJESTRAND 1940). After 5—10 minutes it often closely approached the pre-oxygen level (fig. 1

and 2). When air was again admitted, an increase was regularly observed, even if the ventilation under the influence of oxygen had nearly reached the value before oxygen was given. Thus the ventilation with air often exceeded the previous value for air before the introduction of oxygen. Part of this excess increase subsided in the following minutes.

The inhibitory action of oxygen breathing was regularly observed and could be repeated many times in the normal animal. After vagotomy this reaction was still present, but denervation of the sinus region (with or without preceding vagotomy) abolished it completely. This is shown in fig. 1 C, which also illustrates the fact, previously described by SELLADURAI and WRIGHT (1932)

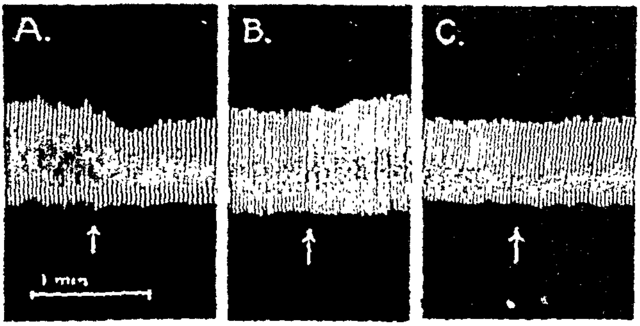

Fig. 1. Cat, decerebrated. Arrows indicate: A, oxygen after air; B, air after oxygen; C, oxygen after air. Between B and C both sinus regions denervated.

and EULER and LILJESTRAND (1936), that sinus denervation reduces the ventilation with an increase in alveolar carbon dioxide tension. (In the experiment of fig. 2 there was no reduction of ventilation after sinus denervation. The gradually developing hyperpnoea in this case probably occurred as the result of central injuries following a prolonged inhalation of 7 per cent oxygen in nitrogen. The figure shows, however, that oxygen breathing is without effect on respiration after sinus denervation.)

There seems to be little doubt that the reduction of respiration caused by oxygen is due to a disappearance of stimulating impulses from the sinus region. The absence of any corresponding decrease in ventilation when the sinus region had been denervated shows that this is practically the sole site of origin of such impulses. This means that, under normal experimental conditions with moderately deep anaestesia, the chemoreceptors in the carotid glomus are permanently stimulated by oxygen want. This is in

complete accord with the results obtained on the action potentials deriving from the chemoreceptors in the anaesthetized cat (EULER, LILJESTRAND and ZOTTERMAN 1939). The rapid change in ventilation when oxygen is given corresponds well to the very prompt changes in the electrical response in the sinus nerve observed in the experiments quoted.

The depression in respiration caused by spontaneous breathing of oxygen is not maintained, as indicated above, either in the cat

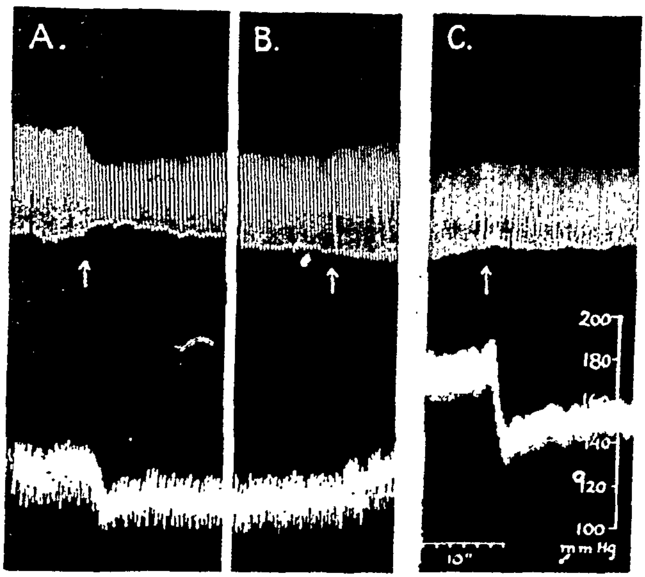

Fig. 2. Dog, chloralose. Upper curves respiration, lower curves blood pressure. Arrows indicate: A, oxygen after air; B, air after oxygen; C, oxygen after air. Between B and C both sinus regions denervated. Vagodepressors intact.

or in the dog. It must therefore be concluded that some other reaction working in a stimulating direction is brought into play. It seems justifiable to assume that one such factor is an increase in the carbon dioxide tension of the blood. During the period of depressed respiration, the carbon dioxide tension rises. After some minutes these factors have to some extent balanced each other, and the ventilation becomes constant at a level slightly below that during air. The "shooting over" observed when oxygen is substituted for air, indicates that the reaction of the regulating mechanisms is more rapid for variations in the oxygen pressure of the blood than for variations in the carbon dioxide. It is possible that the factors mentioned are not the only ones involved in this connection. As pointed out by DUMKE, SCHMIDT and

CHIÓDI (1941), an increased excitability of the center must also be taken into account. Though no direct proof has been given of such an effect, it seems probable from the fact that oxygen breathing after denervation of the glomus is sometimes accompanied by a slight increase in respiration, instead of a decrease such as occurs before denervation. This stimulating effect may be regarded as being due to a relief of the relative insufficiency of the oxygen supply to the centers, accentuated by the diminished ventilation after sinus denervation. The effects described below of oxygen inhalation on blood pressure also speak in this sense. It will be necessary, however, to investigate directly whether similar conditions exist in respect of the effect of oxygen before denervation.

II. Circulation.

a) *Blood Pressure.*

Whereas the respiratory reactions on oxygen inhalation were as a rule very conspicuous, the changes in blood pressure under the same conditions were less obvious. This corresponds well with the observation that a pronounced hypoxia usually only leads to a small increase in blood pressure in the intact animal (EULER and LILJESTRAND 1936, BOUCKAERT, GRIMSON, HEYMANS and SAMAAN 1941).

A typical experiment is given below.

8. 3. 1942. Cat 5.9 kg, anesthetized with 35 ml 1 % solution of chloralose intravenously. Blood pressure and pulse rate recorded from femoral artery. Artificial respiration (after change of gas 3 minutes elapsed before pressure and pulse recording).

Time	Gas breathed	Blood pressure	Pulse rate
0′	air	152	124.5
4′	oxygen	150	121
8′	air	152	124
12′	oxygen	148	119
16′	air	152	124.5
20′	oxygen	150	122.5
Both vagodepressor nerves cut			
30′	air	156	151
34′	oxygen	154	146.5
38′	air	152	149
42′	oxygen	150	145
46′	air	150	144.5
50′	oxygen	148	144.5

Time	Gas breathed	Blood pressure	Pulse rate
Both sinuses denervated			
110'	air	190	115
114'	oxygen	196	115.5
118'	air	182	115
122'	oxygen	192	114.5
126'	air	162	115
130'	oxygen	184	114.5
134'	air	160	115
138'	oxygen	192	117.5

If the artificial ventilation is effected with oxygen instead of air, the blood pressure regularly drops a few millimeters. The effect is obtained also after section of the vagus nerves, but if both sinuses have also been denervated, a rise in the blood pressure is obtained after oxygen, which is much greater than the fall before the denervation. This rise started immediately the oxygen was given, but it took 1—2 minutes before it had reached its full height.

Table 1 gives a summary of similar experiments on 12 cats. With two exceptions, oxygen always caused a fall of the blood pressure. In one of the two exceptional cases the blood pressure remained constant during oxygen inhalation, if the animal breathed spontaneously. During artificial respiration, however, the usual effect was obtained. The other exception showed a small increase of the blood pressure during spontaneous inhalation of oxygen. The diminution of the blood pressure under the influence of oxygen was never great, the maximum being about 10 per cent, on an average it only amounted to 2.2 per cent during spontaneous and 3.4 per cent during artificial respiration.

After vagotomy the effect of oxygen inhalation on the blood pressure was practically unaltered. Denervation of the sinuses alone or after vagotomy was sometimes without any definite influence on the reaction, but it might also lead to a reversal of it, so that a more or less considerable rise of the pressure was observed after oxygen. This is also illustrated by the experiment in fig. 3, which is not included in the table, since the pulse rate was not determined.

In a corresponding experiment on the dog, oxygen inhalation also produced a fall in the blood pressure of the intact animal, but this effect was enhanced by the denervation of both sinuses, as shown in fig. 2.

Table 1.

Percentage Alteration in Blood Pressure and Pulse Rate in Cats, when Oxygen is Respired instead of Air.

s = spontaneous, a = artificial respiration.

Date	Normal animal		After section of vago-depressor nerves		After denervation of both sinuses		After section of vago-depressor nerves and sinus denervation	
	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate
23. 11. 39	s −1.5	−6.2						
25. 11. 39	s −0.8	−3.5						
28. 11. 39	s −9.8	−3.5						
17. 11. 41	s +2.2	−6.1						
9. 1. 42	s −1.4	−1.7			s −5.0	−4.7		
	a −7.6	−2.6			a +7.2	−2.9		
10. 1. 42	s 0	−2.0						
	a −1.7	−5.6						
15. 1. 42	s −2.8	−0.5			s −1.5	−3.1		
	a −3.3	−2.3			a −2.5	−1.1		
21. 1. 42	s −1.3	−4.6						
	a −1.1	−1.4			a +2.0	−0.6	a +1.2	−0.5
26. 1. 42	s −1.4	−0.3	s −2.2	−1.6				
	a −2.1	−2.3	a −7.6	−2.4				
7. 2. 42	s −4.2	−4.6						
	a −5.5	−1.8			a +10.0	−0.4	a +29.0	−0.6
26. 2. 42	a −4.3	−7.6			a +9.4	−1.1	a +14.5	−1.4
8. 3. 42	a −1.6	−2.6	a −1.3	−1.5			a +9.1	[1]+0.4

The simplest explanation of the facts observed seems to be that the slight hypoxemia present in the anesthetized animal breathing air, by its effect on the chemoreceptors of the carotid bodies, not only stimulates respiration, but also the vasomotor center, so that the blood pressure rises. To some extent this is compensated by the baroceptors, and the final result is therefore only a very small increase of the pressure, which is abolished when oxygen is given.

When the vagi are cut, and the sinuses have been denervated, oxygen inhalation can only produce a direct effect on the vasomotor center. As has been shown before (EULER and LILJESTRAND 1936), the blood pressure in the cat after sinus denervation decreases during hypoxemia, and it seems probable that the slight desaturation of the hemoglobin during air breathing may produce a similar effect. It is of interest to point out in this connection that denervation of the sinus mechanism in the cat usually only

[1] −0.1, if last value for oxygen is discarded.

causes a temporary rise in the blood pressure, which is mostly followed by a decrease. In the experiment given in detail on p. 39 a continuous decrease of the blood pressure during air breathing in the denervated animal is observed after the primary rise. It seems reasonable to assume that the oxygen supply in the vasomotor center is somewhat inadequate. Oxygen inhalation will then give a certain relief, and the tone of the vasomotor center is increased again. In the experiment quoted this effect became greater as the pressure fell during air breathing.

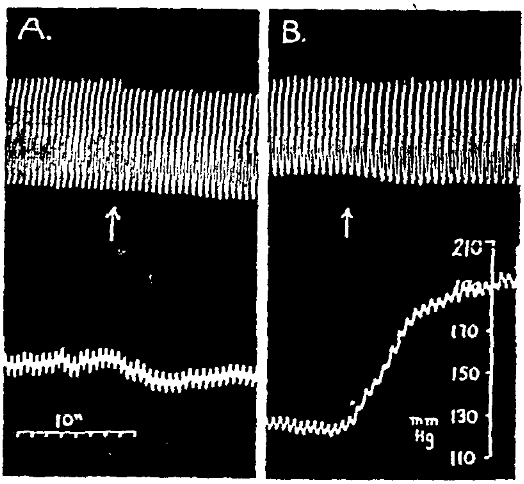

Fig. 3. Cat, chloralose. Upper curves respiration, lower curves blood pressure. At arrows oxygen is breathed instead of air. A, before; B, after denervation of the sinus region.

In the dog the fall in blood pressure observed after oxygen (fig. 2 C) might be explained in a corresponding way, assuming that a relative hypoxia is present during spontaneous breathing of air. After sinus denervation, oxygen gave a greater effect in the same direction as before. That this is due to a previous stimulation of the chemoreceptors of the aortic body seems probable from the facts that hypoxia under these conditions causes a considerable rise in the pressure (EULER and LILJESTRAND 1936), whereas a decrease follows on oxygen inhalation, when the vagi have been cut before or after the sinus denervation (LAMBERT and GELLHORN 1938).

b) *Heart rate.*

Stimulation of the baroceptors of the sinus region influence the pulse rate as well as the blood pressure, and it seems very probable, though it has not been directly proved, that stimulation of the chemoreceptors will increase the heart rate, just as well as it may elicit a rise in blood pressure. Respiration of oxygen would then abolish the effect. As a matter of fact, DAUTREBANDE and HALDANE (1921) observed a slowing of the heart rate during inhalation of oxygen in man.

From Table 1 it is found that a reduction of the pulse rate during oxygen treatment was observed in every case, during spontaneous as well as during artificial respiration. The effect is reversible. The average decrease is 3.3 per cent.

A certain decrease in the oxygen effect is probable after vagotomy or sinus denervation; if both have been performed, most of the action disappears, but a small effect seems still to be left. It must be concluded that the slowing of the pulse rate in consequence of oxygen inhalation is mainly due to the disappearance of the stimulation of the chemoreceptors.

Summary.

When oxygen was substituted for air in anesthetized cats or dogs, an almost instantaneous, sometimes very considerable, reduction of respiratory rate and amplitude occurred. This effect was abolished by denervation of both sinuses and was due to the disappearance of a slight oxygen want which stimulated the chemoreceptors of the carotid body. After some minutes of oxygen breathing the ventilation again closely approximated to the value from air breathing. This compensation is partly explained by an increased carbon dioxide tension, but a greater excitability of the center may also occur.

Oxygen inhalation under the same conditions usually caused a fall in arterial blood pressure, amounting on an average to 2.2 per cent in the cat. After denervation of both sinuses and section of the vagodepressor nerves, oxygen inhalation led to an increased blood pressure. It is assumed that the first effect is due to the abolition by oxygen of the normal stimulation of the chemoreceptors, whereas the second effect is caused by the relief of a central depression, caused by hypoxia.

The pulse rate also decreased, on an average 3.3 per cent in the cat, during oxygen inhalation. Most of the effect was abolished by section of the vagodepressor nerves and denervation of the sinuses.

The expenses of this investigation have been defrayed by a grant from the Therese and Johan Andersson Memorial Foundation.

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Investigations on Fibrinogen.

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Solutions of pure fibrinogen are necessary for the study of the blood clotting process. Such solutions are not only necessary for investigation of the transformation of fibrinogen into fibrin by means of thrombin but are even more necessary for studies on the first phase of the blood-coagulation, i. e., the formation of thrombin. For demonstration and measurement of the thrombin present, a fibrinogen solution is required, and it is therefore necessary that this solution does not contain any substances acting on the thrombin formation.

This was realized already by some of the older authors. Indeed, fibrinogen is the component in the blood-coagulation which previous investigators first tried to isolate in pure form. Especially the well known work by HAMMARSTEN (1879, 1880) laid the basis for such investigations. A review of the work carried out in this first period has been given by MORAWITZ (1905).

The fibrinogen and its transformation into fibrin was investigated more recently by WÖHLISCH and coworkers. WÖHLISCH believes that the formation of fibrin can be looked upon as a specific and enzymatic denaturation of a protein (fibrinogen) and that the thrombin is a specific "fibrinogen denaturase". Concerning these investigations excellent reviews are given by WÖHLISCH (1929, 1940). Also FISCHER (1935) regards the reaction as an denaturation process. Thus the study of the fibrin formation concerns both the chemistry of enzymes and the chemistry of proteins.

As is well known, fibrin is formed when thrombin acts on fibrinogen. Some earlier authors assumed that fibrinogen could be transformed

into fibrin by the action of thrombokinase (thromboplastin). But it has been definitely shown, especially by SMITH, WARNER and BRINKHOUS (1934) and QUICK (1936), that the classical theory of the blood-coagulation mechanism, as suggested by ALEXANDER SCHMIDT, HAMMARSTEN, MORAWITZ, FULD and SPIRO, is correct, and that pure fibrinogen cannot be clotted by a thrombokinase solution containing no thrombin (cf. also FERGUSON (1931)).

Fibrinogen solutions serviceable for such investigations must be free from prothrombin, as in the presence of calcium ions the prothrombin will be transformed into thrombin by the thrombokinase and thus cause the fibrinogen solution to clot. For removal of the prothrombin from the fibrinogen solution, repeated precipitations of the fibrinogen by salts or acids have been used by different authors, but without giving reliable results, (SMITH, WARNER and BRINKHOUS (1934)). Also the improved method by SCHMITZ (1933 b) does not always give a prothrombin-free product causing no clotting on addition of calcium ions and thrombokinase, (FISCHER (1936) and ASTRUP (1939)).

Complete removal of the prothrombin has hitherto been obtained only by employment of an absorbing substance. BORDET, as the first, showed that prothrombin can be removed in this way. In a paper, which is a model of scientific investigation and gives the reader a great pleasure, it is shown that calcium fluoride adsorbs prothrombin from plasma (BORDET and GENGOU (1904)). Other substances have the same ability in a varying degree. BORDET also uses barium sulphate (BORDET and DELANGE (1912)) for the purpose and comes to the conclusion that purified fibrinogen does not react with thrombokinase, and that the conflicting results obtained by some other authors are due to impure fibrinogen solutions (BORDET and DELANGE (1914 a)). In the last-mentioned paper tricalcium phosphate is used for the first time as an absorbent. In the later papers of BORDET this substance is preferred (BORDET and DELANGE (1914 b) and BORDET (1919, 1920). By the absorption also traces of thrombokinase are removed.

Others authors have used the principle of BORDET for obtaining prothrombin-free plasma and fibrinogen. Tricalcium phosphate was used by SUMNER (1922), WIEMER (1929), WÖHLISCH and JÜHLING (1938) and ASTRUP (1938, 1941 a). Also other substances have been used, namely: magnesium hydroxide (FUCHS (1930), SMITH, WARNER and BRINKHOUS (1934)) and aluminium hydroxide (QUICK (1936), FERGUSON (1938)).

Fibrinogen is a rather unstable substance which can easily be denatured by simple physical and chemical measures. Therefore there is no generally accepted method for the preparation of fibrinogen, and almost every investigator has used his own. These methods use two principles for the isolation, either a precipitation by saturated salt solutions or a precipitation after dilution with distilled water and addition of acid. The first-mentioned principle is the most commonly used, especially in the method described by HAMMARSTEN (1879) with employment of 1 vol. of saturated NaCl. A fibrinogen solution prepared according to this method generally is known as Hammarsten fibrinogen. The other principle has been used especially by J. MELLANBY (1909) (Mellanby fibrinogen) but had already been described earlier, cf. HAMMARSTEN (1879).

1. Precipitation of Fibrinogen.

In order to obtain an idea of the conditions for precipitating the fibrinogen, the method of SCHMITZ (1933 a) is used for the qualitative investigation of protein solutions. According to this, increasing amounts of ammonium sulphate are added to the protein solution and the resulting turbidity is measured in a Zeiss Pulfrich nephelometer. SCHMITZ (1933 b) himself used this method for investigation of fibrinogen.

The precipitation is investigated on plasma of horse, ox and chicken to which potassium oxalate or sodium citrate has been added. Also the corresponding plasma from which the prothrombin has been removed by absorption on tricalcium phosphate, as described before (ASTRUP 1938), is investigated. This plasma is called BORDET plasma. For the precipitation, saturated solutions of ammonium sulphate and sodium chloride are used.

The precipitation curves obtained for oxalated plasma, citrated plasma and BORDET plasma from the same species do not show any difference, as the precipitation limits for fibrinogen, globulin and albumin are the same in the three cases. For the different species the precipitation limits are with ammonium sulphate: for horse fibrinogen, 25 per cent saturation; for ox fibrinogen, 30 per cent saturation; and for chicken fibrinogen, 35—40 per cent saturation. Some typical curves are shown in Fig. 1. It is interesting that the apex for maximal precipitation of fibrinogen

is relatively higher for chicken plasma than for horse and ox plasma. Also the differences in the precipitation limits indicate chemical differences in the fibrinogen from different species.

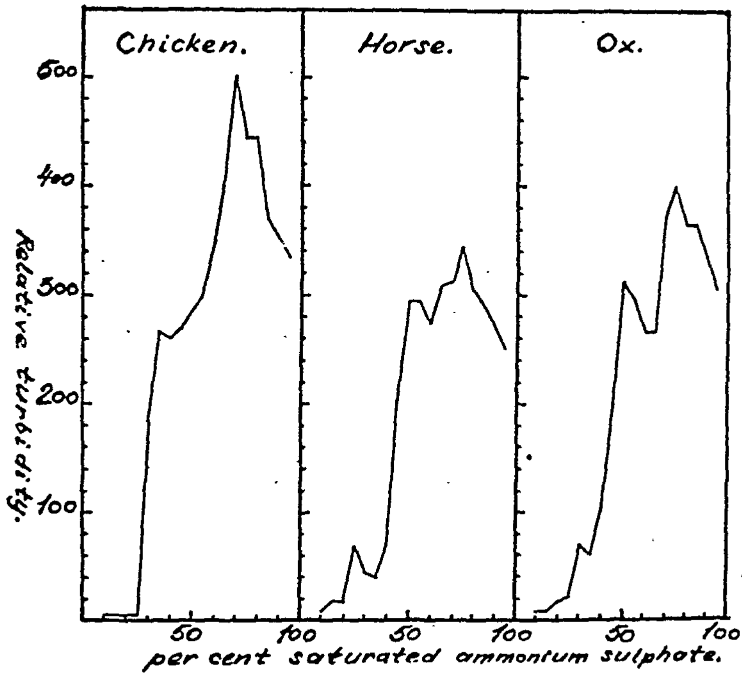

Fig. 1. Precipitation curves of different plasmas using ammonium sulphate: Chicken oxalate plasma, diluted 4 times. Citrate plasma from horse, diluted 5 times. Oxalated plasma from ox, diluted 4 times.

The curves obtained by precipitation with saturated NaCl do not show any maxima. Two such could be expected, namely one for fibrinogen and the other for globulin. Albumin should not give any precipitation with sodium chloride. Curves of the type shown in Fig. 2 are obtained, however.

From these curves the limits for precipitating fibrinogen can be determined only very roughly. Sodium chloride is therefore not suitable for investigations after the method of SCHMITZ. SCHMITZ himself (1937) has investigated precipitation curves of protein by using different salts, and finds, that ammonium sulphate is best suited for the purpose. Surely, therefore, it is not a matter of accident that ammonium sulphate is the salt most commonly used for precipitation of proteins. From the curves it is seen that horse fibrinogen, which, according to HAMMARSTEN, is precipitated by half saturation with sodium chloride, first gives

a considerable turbidity at 0.5—0.7 saturation. This shows the unsuitability of sodium chloride for studies on the precipitation after the method of SCHMITZ — the reasons for this will be discussed later.

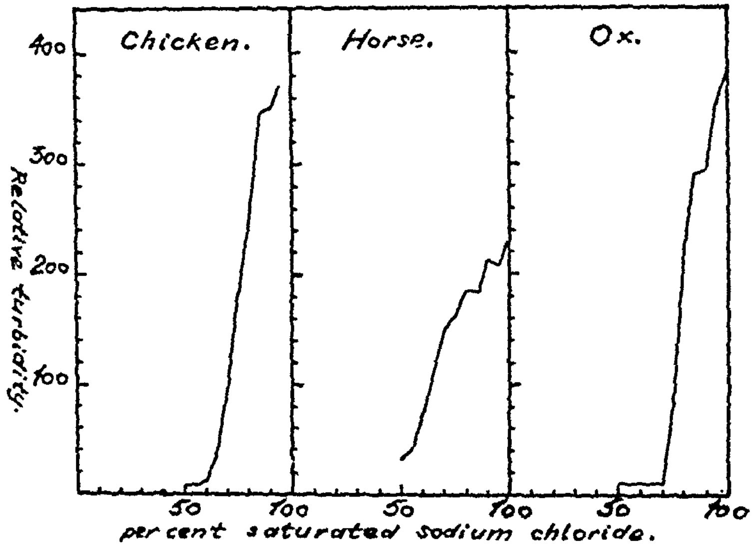

Fig. 2. Precipitation curves of different plasmas using sodium chloride: Chicken plasma. Horse citrate plasma. BORDET plasma from ox.

2. Purification Experiments.

Fibrinogen solutions are now prepared by different methods as described in the literature. Precipitation curves are obtained both with ammonium sulphate and with sodium chloride. Sodium chloride gives just as poor results here as when used on the plasma and shall therefore not be discussed. SCHMITZ, using horse fibrinogen, has made a similar investigation (SCHMITZ (1933 b)) and comes the result that fibrinogen precipitated according to HAMMARSTEN is very impure and does not seem to be amenable to further purification by salt precipitation.

Fibrinogen from horse, ox and chicken is now investigated. Only the experiments with ox fibrinogen will be mentioned, as only this is used for the later experiments. Usually BORDET plasma is used as starting material in order to get rid of prothrombin and thrombokinase.

A. Mellanby Fibrinogen.

Mellanby fibrinogen is prepared from ox plasma as described before (ASTRUP and DARLING (1940, 1941)) after the method of MELLANBY (1931). The resulting product gives the precipitation curves shown in Fig. 3. For comparison also a curve is shown for chicken fibrinogen, prepared as described before (ASTRUP (1938)) according to MELLANBY (1909). The two curves show large differences. While the chicken-Mellanby-fibrinogen seems to be a rather pure fibrinogen, this is not the case with ox fibrinogen which contains large amounts of other globulins. This undoubtedly is due to the different distribution of the proteins in the two plasmas which already has been expressed in the curves in Fig. 1.

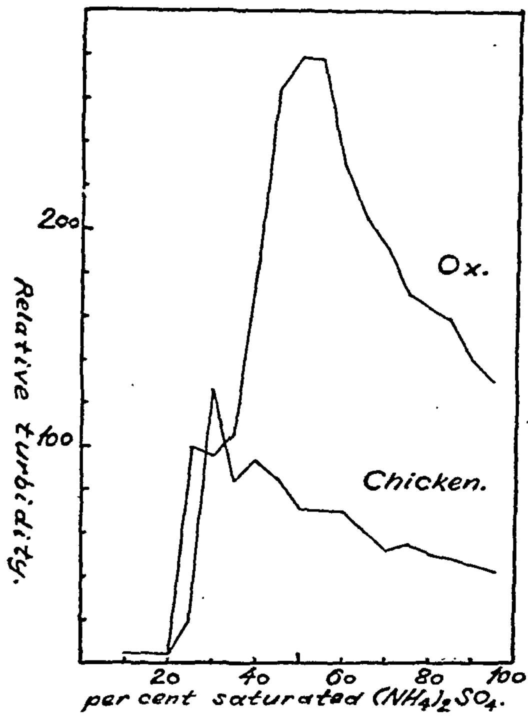

Fig. 3. Precipitation curves of MELLANBY fibrinogens.

The precipitation curves obtained by using Bordet plasma show the same distribution of proteins as the plasma not treated with tricalcium phosphate, which shows the minimal action of the adsorbing agent on the plasma except to remove prothrombin and thrombokinase.

B. Hammarsten-Fibrinogen.

The method of HAMMARSTEN for precipitation of fibrinogen by half saturation with sodium chloride has found extensive use, and since its appearance it has undergone only minor modifications, such as precipitation at low temperature and at a slightly acid reaction, cf. FLORKIN (1930).

It is well known, however, that the method of HAMMARSTEN is best performed on horse plasma, while plasma from other species often gives unsatisfactory results. Especially ox plasma gives poor yields and it is necessary to add more sodium chloride, acid or other salts (cf. MORAWITZ (1904), HUISKAMP (1905) and WÖHLISCH (1924)). According to WÖHLISCH (1925) this is due to differences between the dif-

ferent fibrinogens and not to influences caused by some accompanying substances. The precipitation of various fibrinogens has been investigated by DAVIDE (1925) and was found to be very different (cf. also ASTRUP (1941 b), ASTRUP and DARLING (1942)). HAMMARSTEN himself (1879) mentions that the method can be used on ox plasma, but it must be remembered that in his original investigations he used plasma to which $^1/_3$ vol. of saturated magnesium sulphate solution had been added.

Our investigations also show that ox plasma cannot be precipitated satisfactorily with 1 vol. of saturated sodium chloride solution. By the addition of diluted acid to the mixture, however, a precipitate is formed. The precipitate contains more fibrinogen the more acid has been used. At the same time the content of other proteins is also increased and the purity of the precipitated fibrinogen is decreased. It is possible, however, to find a pH value (i. e. 4.6) where a single precipitation gives both a satisfactory yield and a good purity. The curve of such a fibrinogen is shown in Fig. 4.

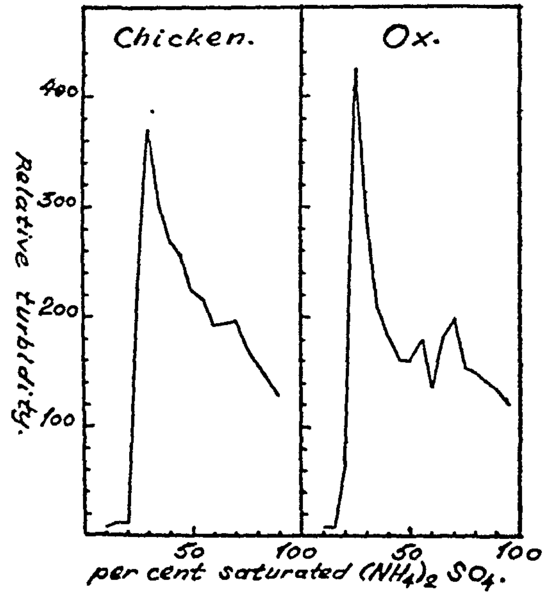

Fig. 4. Precipitation curves of HAMMARSTEN fibrinogens.

On Fig. 4 also the curve for a chicken fibrinogen prepared in a similar way (pH about 6) is shown. The curves show that the fibrinogen solutions are rather pure and the method of HAMMARSTEN seems not to give complicated mixtures as found by SCHMITZ (1933 b).

Fibrinogen solutions were also prepared by first precipitating the plasma according to MELLANBY and then reprecipitate with sodium chloride. Solutions were obtained which according to the precipitation curves and corresponding to the amount of fibrin found by addition of thrombin seemed to be of a greater purity than the preceding solutions.

By further examination of the fibrinogen solutions prepared it is found however that their stability is very poor, and Mellanby fibrinogen seems to be the most unstable. Probably therefore dilution and acidification make the fibrinogen labile. It was also found that the purity of the products obtained was highly dependent on an accurate adjustment of the degree of acidity. Therefore the methods mentioned are not suitable for the precipitation of fibrinogen solutions which are

to be used for further investigation. Products prepared by precipitation by ammonium sulphate were therefore investigated. HAMMARSTEN himself (1880) has noticed the poor stability of acid precipitated fibrinogen.

C. Ammonium Sulphate Fibrinogen.

It is well known that ammonium sulphate is the salt most used for precipitation and fractionation of protein solutions. Also for the precipitation of fibrinogen it has been much used, and it has the advantage that fibrinogen can be precipitated in plasma from different species at about the same concentration of the salt and at neutral reaction.

WÖHLISCH (1940) recommends for precipitation of ammonium sulphate fibrinogen the method described by McLEAN (1920). McLEAN worked with dog plasma and obtained good results by precipitation with 0.25 vol. saturated ammonium sulphate (20 per cent saturation). According to HUDEMANN (1940) this method, used on horse and ox plasma, gives a fibrinogen which is so pure that it is unnecessary to remove prothrombin by absorbtion. In our studies on ox fibrinogen we have also tried the method described by McLEAN, but without obtaining satisfactory results as only a fraction of the fibrinogen is precipitated.

A precipitation with 28 per cent saturation gave good results. Fig. 5 shows the precipitation curve for such a fibrinogen solution prepared from Bordet plasma from ox by precipitation at 0.28 saturation with ammonium sulphate and reprecipitation. It is seen that the curve shows a very satisfactory appearance which suggests the presence of only very small amounts of other proteins.

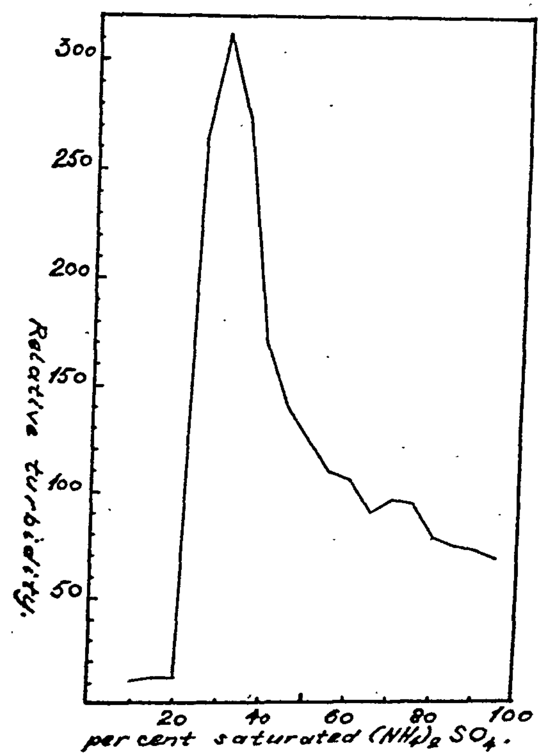

Fig. 5. Precipitation curve of fibrinogen prepared by ammonium sulphate precipitation.

The fibrinogen just prepared showed greater stability than the aforementioned preparations, and the method was therefore used for the final preparation of fibrinogen. The greater stability of

the ammonium sulphate-precipitated fibrinogen is in accordance with the experiences of FERGUSON and ERICKSON (1939), while v. FARKAS and GROÁK (1929) find that sodium chloride produces less denaturation. It is to be mentioned, however, that the first mentioned authors worked with dog plasma and the last-mentioned with human plasma.

3. Preparation of Ox Fibrinogen.

Based on the preceding investigations and later experiences the preparations of ox fibrinogen then is as follows:

A. Bordet Plasma.

To 40 ml of a 10 per cent solution of $CaCl_2$ sicc. are added 40 ml of a 10 per cent solution of trisodium phosphate. After stirring it is centrifuged for two minutes. The precipitate is then washed on the centrifuge three times with distilled water and then three times with physiological NaCl. Then 32 ml of physiological NaCl are added and the precipitate suspended herein.

25 ml of the suspension are added to 150 ml of oxalated ox plasma which is made neutral on litmus paper by adding 1-n HCl. After standing for 15 minutes with occasional stirring it is centrifuged and, after neutralization, 25 ml of the suspension are again added to the supernatant. After standing for one hour with occasional stirring it is again centrifuged and neutralized, (pH 7.0—7.5). The resulting Bordet plasma is kept at 0°.

B. Ox Fibrinogen.

To 100 ml of Bordet plasma 150 ml of distilled water are added and the fibrinogen is then precipitated with 100 ml of saturated ammonium sulphate. All solutions are cooled to 0°. The addition of ammonium sulphate is carried out slowly through a funnel with its tip under the surface of the plasma solution and with stirring. After 15 minutes the mixture is centrifuged. The supernatant fluid is poured off, and the remaining solution is removed by a strip of filter paper. The precipitate is then dissolved in 50 ml of physiological NaCl and after centrifugation, in order to remove some insoluble material, diluted to 100 ml with distilled water. It is again precipitated with 40 ml of saturated ammonium sulphate and after centrifugation the precipitate is dissolved in 45 ml of 2 per cent NaCl. The solution is centrifuged and then dialysed in a cellophan casing for 24 hours at 0° against 2 per

cent sodium chloride containing 0.1 per cent potassium oxalate. *The fibrinogen solution then gives practically no reaction with* Nesslers reagent. It is kept at 0° in the cellophan casing, and before its use, it is diluted 3—5 times with physiological sodium chloride, distilled water or buffer solution.

This fibrinogen solution is as a rule stable for 10—14 days when kept in the dialyzing membrane surrounded by 2 per cent oxalated sodium chloride. It odes not clot by addition of $CaCl_2$ and thrombokinase. The absorption and precipitation must be carried out at about neutral reaction in order to obtain stable and reliable preparations. If possible, the ox plasma should not be used fresh but after standing for 24 hours before the preparation of Bordet plasma, as a thrombokinase-containing precipitate is formed, which can be discarded after centrifugation. When using chicken plasma (ASTRUP (1938)) tricalcium phosphate was only added once, but when oxalated ox plasma was used, two separated additions of tricalcium phosphate gave better results. This is possibly due to the content of oxalate. By dilution of the Bordet plasma with water before the precipitation, the precipitation of other proteins is decreased so that a purer product is obtained. The tricalcium phosphate should be fresh precipitated (cf WIEMER (1929)). Prothrombin cannot be removed from a fibrinogen solution by adding tricalcium phosphate, as the fibrinogen under these conditions is also partially adsorbed. The same phenomenon has also been noticed in the case of aluminium hydroxide by FERGUSON and ERICKSON (1939). Using magnesium hydroxide, however, it seems possible to remove prothrombin from purified fibrinogen solutions, SEEGERS, SMITH, WARNER and BRINKHOUS (1938). The conditions for adsorbing prothrombin are therefore not the same in plasma as in solutions of fibrinogen. The presence of salts (2 per cent NaCl) increases to a high degree the stability of the fibrinogen solutions. This was mentioned by WÖHLISCH (1924) and by v. FARKAS and GRÓÁK (1929) and is used by THORDARSON (1940) for preparation of fibrinogen solutions.

4. Fibrinogen and Thrombin.

The ability of the fibrinogen solutions to react with thrombin determines the usefulness of the prepared solution. Previously we have measured the potency of thrombin by its ability to clot oxalated ox plasma (ASTRUP and DARLING), (1940, 1941)). Subsequently, however, it was found that plasma is not suitable for such measurements, as the individual variations are too large and the relationship is not always linear. Therefore it has been necessary to choose a thrombin preparation as standard for the comparison and measurement of other thrombin preparations. The potency of the standard so chosen was put at 12,000 thrombin

units (T. U.) per g of substance. Fibrinogen solutions show some other advantages for the measurement of thrombin compared with plasma, as it is easier to determine the accurate clotting time, and as it reacts more readily with thrombin. NOLF (1919) has already drawn attention to this point. SEEGERS, BRINKHOUS, SMITH and WARNER (1938) measure the potency of their thrombin preparations on fibrinogen (cf. also SEEGERS (1940)).

The action of thrombin on fibrinogen prepared according to MELLANBY was compared with the action on fibrinogen precipitated by ammonium sulphate. The measurements were carried out in principle as described before (ASTRUP and DARLING, (1941)). In a clotting tube 0.10 ml of a thrombin solution was placed, then 1.0 ml of ice-cold fibrinogen solution was added and the mixture was placed in a water bath at 37°. The clotting moment is the moment when the formation of fibrin is first observed. This moment can be very accurately determined when the tube is gently shaked. Exact observation of the formation of fibrin strands requires the use of a transparent water bath. The measurements show, that there is a considerable difference in the action of thrombin on MELLANBY fibrinogen and on ammonium sulphate-precipitated fibrinogen. When a curve is drawn with the concentration of thrombin as abscissa and the reciprocal of the clotting time as ordinate, Mellanby fibrinogen gives no linear relationship but a curved line. For ammonium sulphate-precipitated fibrinogen a straight line passing through zero is found. Only with the last mentioned fibrinogen the concentration of thrombin and the clotting time are inversely proportional. The cause of this is possibly that the Mellanby fibrinogen, as shown in the earlier mentioned precipitation curves, contains considerable amounts of other proteins (globulins). In Fig. 6 examples of such curves are shown. The amount of thrombin expressed in per cent of the original solution is given as abscissa with the clotting time expressed in reciprocal minutes as ordinate.

From the curves it is seen that Mellanby fibrinogen is unsuitable for measurement of thrombin activity while the ammonium sulphate precipitated fibrinogen is very suitable.

The determination of the potency of a thrombin preparation is then carried out as follows:

50 mg of the thrombin used as standard are dissolved in 15 ml of physiological NaCl with addition of one drop of octyl alcohol. The solution contains 40 thrombin units (T. U.) per ml. By

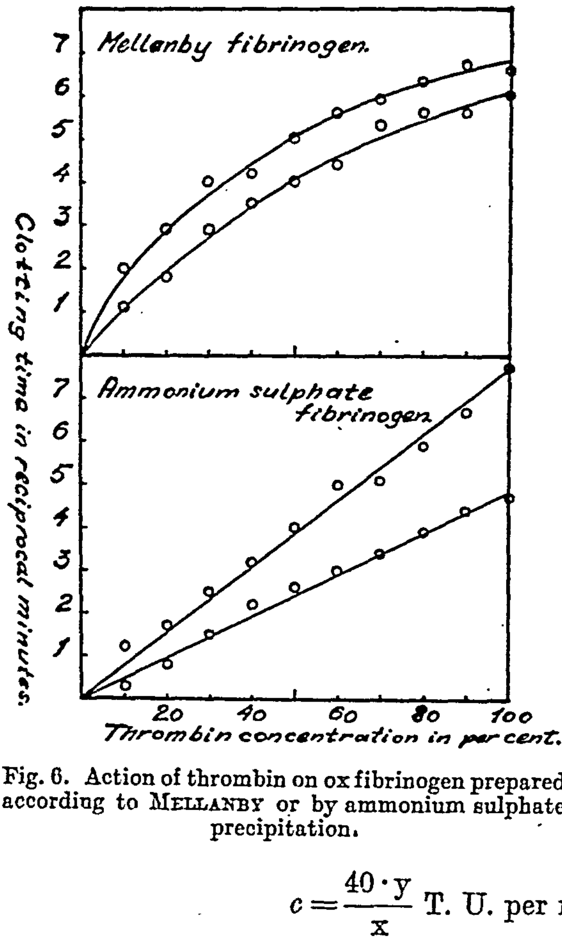

Fig. 6. Action of thrombin on ox fibrinogen prepared according to MELLANBY or by ammonium sulphate precipitation.

diluting this thrombin solution a curve, as shown in Fig. 6, is determined in the usual way. After drawing a straight line through the points on the curve, its slope is determined (by measuring the ordinate for the original solution, i. e., 100 per cent thrombin) in reciprocal minutes (x). In the same way a curve for the unknown thrombin solution is determined, and the corresponding slope measured (y reciprocal minutes). The potency c of the unknown solution is found from the equation

$$c = \frac{40 \cdot y}{x} \text{ T. U. per ml}$$

As the clotting of fibrinogen is very sensitive for even small alterations of the pH value and of the salt concentration of the solution (ASTRUP 1941 b)), it is not possible with satisfactory accuracy to set up any definite thrombin standard from the clotting time of a fibrinogen solution. It is fortunate, therefore, that a dry preparation of thrombin has shown a very good durability. Such a preparation has now been used for almost two years without any deterioration. The use of fibrinogen solutions for determination of thrombin has been discussed by ATZLER (1929).

5. The Solubility of Fibrinogen.

The solubility of fibrinogen has in particular been investigated by FLORKIN (1930). He uses horse fibrinogen which he dissolves in potassium chloride, potassium phosphate and ammonium

phosphate. From the solubility curves he draws the conclusion, that fibrinogen is a homogeneous substance. Fibrinogen was considered a single, well-defined substance, until investigations by v. FARKAS and GROÁK (1929) and KYLIN and PAULSEN (1936) seemed to show that different fibrinogens existed in blood plasma. STENHAGEN (1938), however, using the TISELIUS method for electrophoretic investigations, found only one single definite substance. Also the precipitation curves (fig. 4 and 5) found in the present investigations suggest that it is a single component, though by these curves it is not possible to distinguish between closely related substances.

In fig. 1 and 2 there was a remarkable difference between the character of the ammonium sulphate curves and the sodium chloride curves, as the last-mentioned curves gave no definite limits for the precipitation. In the determinations, 10 ml of salt solution was used to 0.1 ml of a more or less diluted plasma. The amount of proteins was therefore slight in relation to the large amount of precipitating solution. Even a low solubility of the proteins in the sodium chloride solution will suffice to explain the poor results. The solubility of fibrinogen in half saturated sodium chloride was therefore investigated. In a series of tubes 5 ml of ox plasma are pipetted. Varying amounts of water and of saturated sodium chloride are added so that the mixture always is 0.5 saturated with NaCl. The precipitated fibrinogen is removed by centrifugation and dissolved in 2 ml of distilled water. A definite amount of a thrombin solution is added, and the mixture stands at 0° untill the next day, when the fibrin formed is removed. The precipitated fibrinogen is then determined as the difference between the total nitrogen content of the solution and the nitrogen content after removal of fibrin. The results are shown in Table 1.

Table 1.

Plasma	H_2O	Saturated NaCl	Total-N	Rest-N + Thrombin-N	Thrombin-N	Fibrinogen-N
ml	ml	ml	mg	mg	mg	mg
5.0	0.0	5.0	2.674	2.312	0.074	0.486
5.0	5.0	10.0	1.430	1.190	0.074	0.314
5.0	10.0	15.0	0.824	0.685	0.074	0.213
5.0	15.0	20.0	0.448	0.418	0.074	0.109

From this Table it is seen that the amount of precipitated fibrinogen shows a decrease, proportional with the increasing amounts of the solution, to such an extent that the amount of fibrinogen dissolved under the experimental conditions is large in proportion to the total amount of fibrinogen. Obviously it is therefore impossible to obtain good precipitation curves by using sodium chloride solutions, as the conditions for precipitation of fibrinogen here are even more unfavourable. It is evident, that it is necessary when judging precipitation curves of proteins obtained according to SCHMITZ, to take into consideration a possible solubility of the protein in the precipitating salt solution. Ammonium sulphate appears to offer the most favourable conditions for precipitation.

Summary.

1. The precipitation limits for fibrinogen have been studied under various conditions.

2. The purification of fibrinogen solutions has been investigated and a method for the preparation of a purified prothrombin-free fibrinogen solution from ox plasma is described.

3. The measurement of thrombin by employment of ammonium sulphate precipitated fibrinogen has been described. Mellanby fibrinogen is unsuitable for measuring of thrombin.

4. Fibrinogen shows rather high solubility in half-saturated sodium chloride.

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From the Pharmaceutical State Laboratory, Stockholm.

The Influence of Ergotamine on the Action of Ephedrine and Sympatol on the Isolated Rat's Intestine.

By

BÖRJE EMILSSON.

(Received 27 February 1942.)

It has been shown (EMILSSON 1942) that previous addition of ergotamine does not abolish the inhibiting action of ephedrine and sympatol on the isolated rabbit's small intestine. As BENIGNI (1930—31) and AIAZZI-MANCINI (1930—31) claim to have shown that ergotamine inverts the inhibiting action of ephedrine and abolishes that of sympatol on the isolated small intestine of Mus Rattus there would seem to exist a fundamental difference in the mode of reaction towards these drugs of the intestine of the rabbit and the rat. Therefore, it may be justified to reinvestigate the influence of ergotamine on the action of ephedrine and sympatol on the isolated rat's intestine.

Literature.

AIAZZI MANCINI (loc. cit.) reports that sympatol inhibits the isolated small intestine of the rat (Magnus' technique, Ringer-Locke's solution) in conc. 30—100 γ/ml (considerable decrease of tone, diminution of amplitude or complete paralysis of the pendulum movements). Previous addition of ergotamine tartrate completely abolishes the effect.

One of AIAZZI-MANCINI's experiments may be quoted in some detail: 50 γ/ml sympatol cause considerable fall of tone and complete paralysis of the pendulum movements. After a while 1 γ/ml

ergotamine tartrate is added (*no washing before the addition!*) whereupon tone and amplitude gradually return to the initial state. Upon the repeated addition of 50 γ/ml sympatol no inhibiting effect is obtained.

The author concludes that sympatol has a pure sympathicotrop action.

According to BENIGNI (loc. cit.) ephedrine stimulates the isolated small intestine of Mus Rattus in conc. 5—10 γ/ml (increase of tone, amplitude unaffected or decreased.) Conc. 40—100 γ/ml have an inhibiting effect similar to that of adrenaline, whereas conc. about 20 γ/ml are inactive or exert a very weak action. The motor action of ephedrine in conc. 10 γ/ml is not affected by previous addition of ergotamine (1 γ/ml), while the inhibiting action of larger doses of ephedrine is reversed into a motor effect.

The author concludes that ephedrine partly stimulates the smooth muscle, partly has a sympathicotropic effect. The latter is manifested only by large doses of ephedrine.

Methods.

The experiments were performed on the isolated small intestine of Mus Rattus according to the Magnus technique. The intestinal segments were suspended in Tyrode solution at a temp. of 38° C. Through the solution was bubbled a mixture of 95 % O_2 and 5 % CO_2.

The following drugs were used:

dl-ephedrine hydrochloride (Bayer), dl-sympatol hydrochloride (Boehringer) and ergotamine tartrate (Sandoz). The conc. of ephedrine and sympatol are calculated as γ/ml free base.

Results.

The isolated small intestine of the rat is inhibited by sympatol in conc. higher than 50 γ/ml (considerable fall of tone, often complete paralysis of the pendulum movements). The effect is rather difficult to wash out, 2—4 washings with fresh Tyrode solution being necessary for complete restoration of the mobility of the intestine. In most cases the reproduction of the effect is quite feasible, occasionally the reactivity of the intestine may diminish upon the repeated addition of sympatol.

In most cases previous addition of ergotamine (0.4—14 γ/ml) does not diminish the inhibiting action of sympatol (70—140 γ/ml). (See fig. 1.) Occasionally the inhibiting effect is considerably

decreased but even after repeated washings during 2 hours the control effect always is still weaker. Therefore, the explanation must be found in the diminished reactivity of the intestine towards sympatol, which may arise in the course of the experiment (compare above!).

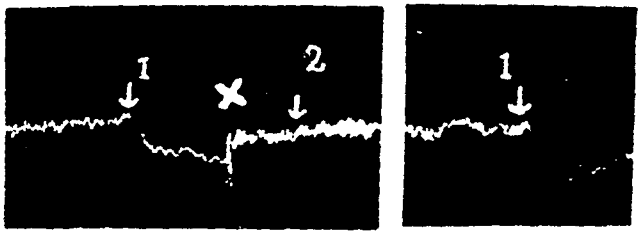

Fig. 1. Isolated rat's intestine in Tyrode solution.
1. = 70 γ/ml dl-sympatol.
2. = 8.6 γ/ml ergotamine.
x = washing thre times.

It was quite easy to make an exact reproduction of the one of Aizzi Mancini's experiments quoted above. But if the same experiment is performed *without any addition of ergotamine* no inhibiting effect upon the second addition of sympatol is observed either. (See fig. 2.) The explanation is quite simple: if the first dose of sympatol is not washed out, the intestine becomes tachyphylactic and does not react to sympatol any longer.

Fig. 2. Isolated rat's intestine in Tyrode solution.
1. = 70 γ/ml dl-sympatol.

In conc. less than 1,000 γ/ml ephedrine in most cases does not affect the isolated small intestine of Mus Rattus, in some cases stimulation is observed. Conc. higher than 1,000 γ/ml have a weak or moderate inhibiting action (mainly decrease of tone). The sensitivity of the intestine towards ephedrine considerably decreases after the first dose and therefore in most cases it is impossible to reproduce the effect with full strength.

The inhibiting action of ephedrine in conc. higher than 1,000 γ/ml is considerably decreased by previous addition of ergotamine (0.4—1.4 γ/ml). But after repeated washings during 1 hour the control effect always is still weaker. Therefore, the diminution of the inhibiting effect must depend on the decreased sensitivity of the intestine towards ephedrine (tachyphylaxis).

Discussion.

From the experiments it may be concluded that previous addition of ergotamine does not affect the inhibiting action of sympatol and ephedrine on the isolated small intestine of the rat. The observed diminution of the inhibiting effect after the first dose of the drug (occasionally in the case of sympatol, almost always in the case of ephedrine) depends on the fact that the intestine may become tachyphylactic against these drugs and is in no way due to any antagonizing effect by ergotamine.

In accordance with the views, which have been put forward in a previous paper (EMILSSON, 1942), it may be concluded that the inhibiting action of sympatol and ephedrine on the isolated rat's small intestine is due to *direct muscular depression* and hears no relation to the sympathetic nerve endings or receptors.

Summary.

1. The action of sympatol and ephedrine on the isolated small intestine of the rat and the influence by previous addition of ergotamine on this action have been studied.

2. Sympatol inhibits the intestine in conc. 60—120 γ/ml, ephedrine in conc. higher than 1,000 γ/ml. In conc. less than 1,000 γ/ml ephedrine in some cases stimulates the intestine.

3. Previous addition of ergotamine does not affect the inhibiting action of sympatol and ephedrine.

4. It is concluded that the inhibiting action of sympatol and ephedrine on the isolated small intestine of the rat is due to direct muscular depression.

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From the Pharmaceutical State Laboratory, Stockholm.

The Influence of Sympathicolytica on the Action of Adrenaline Substitutes on Isolated Intestine.

II. Yohimbine and Hydrastinine.

By

BÖRJE EMILSSON.

(Received 27 February 1942.)

In a previous paper (EMILSSON 1942) the influence of bee venom and ergotamine on the action of a number of adrenaline-like drugs on isolated intestine has been treated. The investigation now has been extended to include yohimbine and hydrastinine also.

WEGER (1927 and 1935—36) reports that low conc. of yohimbine often stimulate the isolated rabbit's small intestine (increase of tone) but this stimulation rapidly changes into a more marked inhibition of the intestine. Higher conc. immediately inhibit the intestine (decrease of tone and amplitude). Previous addition of yohimbine in conc. 0.01—20 γ/ml decreases or abolishes the inhibiting action of adrenaline (0.01—0.03 γ/ml). After repeated washings the normal reactivity of the intestine towards adrenaline is rapidly restored if the conc. of yohimbine was low, if the conc. was high the reactivity is restored slowly and uncompletely.

BOSCHETTI and COZUTTI (1936) find that yohimbine always has a motor action on isolated intestine (increase of tone and amplitude) and that the inhibiting action of adrenaline is abolished by yohimbine. No details are given and therefore the report is difficult to evaluate.

LUNDBERG (1925) reports that hydrastinine stimulates the isolated rabbit's small intestine in conc. higher than 30 γ/ml

(moderate or great increase of tone, the amplitude is unaffected or somewhat increased). *Conc.* higher than 500 γ/ml depress the intestine. Previous addition of sufficiently large doses of hydrastinine completely abolishes the inhibiting action of adrenaline; 250 γ/ml hydrastinine for instance, annul the inhibiting action of 0.0125 γ/ml adrenaline. The author concludes that hydrastinine paralyzes the inhibiting sympathetic nerve-end organs.

Methods.

The experiments were performed on isolated rabbit's small intestine in Tyrode solution according to the Magnus technique. In addition to the drugs employed in part I of this investigation were used yohimbine hydrochloride and hydrastinine chloride Merck.

Results.

In many cases yohimbine in conc. 0.2—7 γ/ml does not affect the motility of the isolated rabbit's intestine, but especially in conc. higher than 4 γ/ml it may have an inhibiting action (decrease of tone or amplitude or both). Under the influence of the highest conc. of yohimbine the movements of the intestine occasionally may become irregular.

Previous addition of yohimbine (0.2—7 γ/ml) decreases or abolishes the inhibiting action of adrenaline (0.03—0.08 γ/ml). After repeated washings with fresh Tyrode solution the normal reactivity of the intestine towards adrenaline is in most cases completely restored, if the conc. of yohimbine was high the reactivity occasionally may be irreversibly diminished. Especially if the dose of yohimbine was high the inhibiting effect of adrenaline after washing is followed by a secondary stimulation of the intestine (motor wash effect).

The inhibiting action of dl-arterenol (0.1—0.3 γ/ml), dl-adrenalone (0.5—3 γ/ml) and l-adrianol (0.3—0.8 γ/ml) is decreased or abolished by yohimbine in conc. 0.2—7 γ/ml (Fig. 1). There seems to be no difference between these drugs and adrenaline as to the dose of yohimbine necessary for complete annulment of an inhibiting effect of given strength. Motor wash effects are particularly frequent in experiments with l-adrianol.

Yohimbine (0.2—7 γ/ml) either does not affect or decreases the inhibiting action of l-corbasil (0.1—0.3 γ/ml). Exceptionally and by employing the highest conc. of yohimbine the inhibiting action may be completely abolished.

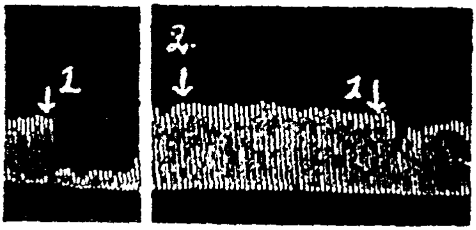

Fig. 1. Isolated rabbit's intestine in Tyrode solution.
1. = 2 γ/ml dl-adrenalone.
2. = 3.4 γ/ml yohimbine.

The inhibiting action of l-, d- and dl-ephedrine (200—1,000 γ/ml), l- and dl-sympatol (100—1,200 γ/ml), suprifen (100—250 γ/ml) veritol (180—250 γ/ml) and benzedrine (80—130 γ/ml) is in no way affected by previous addition of yohimbine in conc. up to 7 γ/ml. (Fig. 2.)

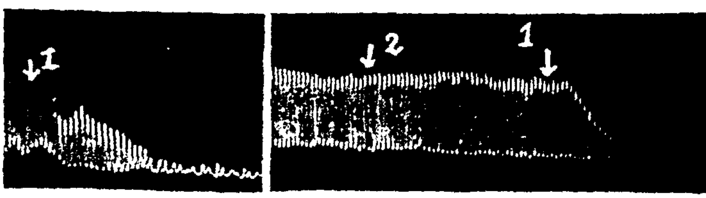

Fig. 2. Isolated rabbit's intestine in Tyrode solution.
1. = 100 γ/ml benzedrine.
2. = 6.2 γ/ml yohimbine.

Hydrastinine in conc. 70—350 γ/ml almost always causes a moderate or great increase in the tone of the isolated rabbit's small intestine. Occasionally the amplitude is unaffected but in most cases it is decreased; the pendulum movements often become rather irregular. Higher conc. (about 500 γ/ml) often have an inhibiting action.

Previous addition of hydrastinine (100—350 γ/ml) considerably decreases or completely annuls the inhibiting action of l-adrenaline (0.03—0.08 γ/ml), dl-arterenol (0.1—0.3 γ/ml), dl-adrenalone (0.5—3 γ/ml) and l-adrianol (0.3—0.8 γ/ml) (Fig. 3). After repeated washings with fresh Tyrode solution the normal reactivity of the intestine is in most cases restored. Motor wash effects are rather frequent especially in the experiments with l-adrianol.

The inhibiting action of l-corbasil (0.1—0.3 γ/ml) is decreased by hydrastinine in conc. 100—350 γ/ml; in exceptional cases the action is completely abolished.

Hydrastinine in conc. up to 450 γ/ml in no way diminishes the

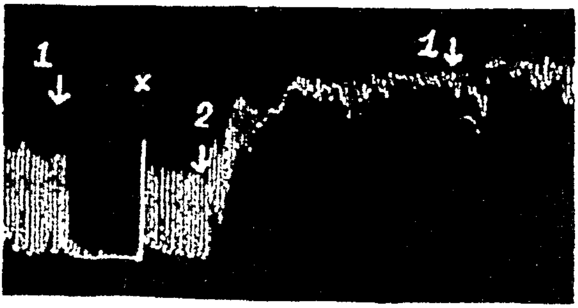

Fig. 3. Isolated rabbit's intestine in Tyrode solution.
1. = 0.2 γ/ml dl-arterenol.
2. = 250 γ/ml hydrastinine.
x = washing.

inhibiting action of l-, d- and dl-ephedrine (200—1,000 γ/ml), l- and dl-sympatol (100—1,200 γ/ml), suprifen (100—250 γ/ml), veritol (180—250 γ/ml) and benzedrine (80—170 γ/ml). In contrariety the inhibitory effect in several cases seems to be intensified by previous addition of hydrastinine. (Fig. 4.)

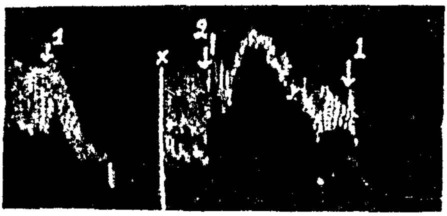

Fig. 4. Isolated rabbit's intestine in Tyrode solution.
1. = 620 γ/ml dl-ephedrine.
2. = 310 γ/ml hydrastinine.
x = washing three times.

Discussion.

Yohimbine and hydrastinine antagonize the action of adrenaline on a variety of organs and tissues included the isolated rabbit's small intestine. Therefore, they may be classified as sympathicolytica just as ergotamine and bee venom.

However, the results of the combined experiments with yohimbine, hydrastinine and adrenaline-like drugs on isolated rabbit's intestine are not quite so easy to interpret owing to the relatively strong influence on the motility of the intestine, which these sympathicolytica exert in doses necessary for complete annulment of the inhibiting action of adrenaline. Yet it is quite evident that the classification of adrenaline-like drugs which has been made on the

basis of the experiments with bee venom and ergotamine (EMILSSON 1942) is valid also for yohimbine and hydrastinine. The classification is as follows:

I. adrenaline, adrenalone, arterenol, adrianol, corbasil.

The inhibiting action of these drugs is diminished or abolished by previous addition of bee venom, ergotamine, yohimbine or hydrastinine.

II. ephedrine, suprifen, sympatol, veritol, benzedrine.

Previous addition of bee venom, ergotamine, yohimbine or hydrastinine even in maximal doses does not abolish the inhibiting action of the drugs belonging to this group.

Thus, the theory that the inhibiting action of the drugs belonging to group II is due to *direct muscular depression* is further supported by the present experiments. The finding that hydrastinine in some cases intensifies the inhibiting action of these drugs points in the same direction.

Summary.

1. The inhibiting action of adrenaline, adrenalone, arterenol, l-adrianol and l-corbasil on isolated rabbit's small intestine is diminished or abolished by previous addition of yohimbine or hydrastinine in sufficient doses.

2. Previous addition of yohimbine or hydrastinine even in maximal doses in no way diminishes the inhibiting action of l-, d- and dl-ephedrine, l- and dl-sympatol, suprifen, veritol and benzedrine. In contrariety hydrastinine sometimes intensifies the action of these drugs.

3. Thus, the classification of adrenaline-like drugs as to the influence of sympathicolytic drugs on their inhibiting action on isolated rabbit's intestine, which have been made previously on the basis of experiments with bee venom and ergotamine, is valid for yohimbine and hydrastinine also.

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From the University Museum of Normal Anatomy and from the University Institute of Hygiene, Copenhagen.

Observations on Lowered Resistance to Spontaneous Infection Resulting from Sexual Abstinence.

By

ERIK ANDREASEN.

(Received 30 March 1942.)

Since 1934, AGDUHR has published a number of papers showing that the normal sexual functions as displayed in the intercourse between the two sexes in mice and rats induce an increase in the resistance of the organism to toxic factors of diverse nature (large doses of activated ergosterol, various metal salts, alcohols, narcotics, paratyphoid cultures, and diphtheria toxin).

The observations reported in the following throw some additional light on this interesting biological phenomenon, as they seem to show that sexual abstinence in the rat gives rise to the appearance of infected excoriations or ulcers that are not seen in rats allowed the possibility of a normal sexual life. This observation was made in connection with a fairly comprehensive study of the thymolymphatic system in normal albino rats (mus norvegicus albinus).

The animals here observed originated from the breeding colony of the State Vitamin Laboratory, Copenhagen. All the animals have been treated in the same way: removed from the mother at the age of 28 days and placed in cages of galvanized wire screen (measuring 15 × 20 × 27 cm.), in which they were kept continually in solitary confinement. These cages were standing on high legs, so that the feces dropped down through the meshes of the floor and coprophagy was excluded. The cages were standing in a clean, dry room with frosted window-panes shutting out

any direct sunlight. The temperature was kept constantly at a level of 22° C., summer and winter.

The diet given these animals has been the same as employed since 1926 for breeding animals of the rat colony. Altogether 22 generations have been bred on this diet.

When given ad libitum, the diet is adequate quantitatively as well as qualitatively. The animals thrive on it and their growth is normal. The composition of the diet, which is kept constant throughout the year, is as follows:

Skimmed milk powder	30 %
Rice flour	40 %
Yeast	15 %
Coconut oil	14.7 %
Cod liver oil	0.3 %

The skimmed milk powder is prepared from pasteurized milk; its composition is: protein 37.5 %, lactose 48.5 %, fat 1 %, mineral salts 9 %, water 4 %.

The rice flour is a mixture of 85 % ordinary commercial rice flour (made from polished rice) and 15 % rice husks (ground to meal). The rice flour contains 78 % carbohydrate, 7 % protein, and 0.5 % fat. The rice husks contain 47—57 % carbohydrate, 10—14 % protein, and 10—14 % fat.

The yeast is autolyzed tub yeast containing 16 % protein, 6 % carbohydrate, and 1 % fat.

The coconut oil (palmin) is 100 % fat of the following composition: fatty-acids (lower than C_{12}) about 15 %, lauric acid 45—51 %, myristhinic acid 17—20 %, palmitic acid 4—8 %, stearic acid 1—5 %, oleic acid 2—10 %, linoleic acid 1 %.

This diet was originally composed by Gudjónsson (1930) and employed by him in his experimental studies on vitamin A. On such a diet, the young rats will, at the age of 28 days (weaning time) be equipped with small but uniform depots of vitamin A. The vitamin A supply to the animals is given chiefly in the cod liver oil, which contains about 2,000 I. U. per g.; and this is sufficient for prevention of avitaminotic conditions and to ensure a normal growth.

Also the vitamin E depots are rather small in animals kept on this diet (Ringsted 1936), though fully sufficient to preserve the fertility. Vitamin E is supplied chiefly through the cod liver oil and coconut oil.

The animals receive a plentiful supply of vitamins B_1 and B_2 through the yeast, and vitamin D through the fats. The rats are able themselves to synthesize vitamin C.

Mineral salts are supplied through the milk powder. The animals have free access to food and water throughout the 24 hours.

Among the many animals (500—600) employed by me since 1938 for experimental studies, I have continually found some in-

dividuals presenting pathological processes in the skin. These processes have shown the form of small, crust-covered excoriations localized to the head, neck and forefeet. Often these excoriations have been no larger than pinheads; but sometimes they have been confluent, forming large ulcers that were visible even at a long distance. In every instance these ulcerations have been associated with hyperplasia of the regional lymph glands. The lesion makes its appearance nearly always about the time for sexual maturity (in the 3' month of life), and it has been ascertained in about 30—40 % of the animals, preferably in the males, which also showed the more severe form of the lesion. As a rule, however, the ulcers are so small that their presence is revealed only by a very thorough examination.

In about a dozen animals I have followed the course of the affection from its first appearance, at the age of 3 months, till the animals were 1 year old. Throughout this period the skin lesion remained of practically the same extent. As such ulcers often are observed in old animals too, the lesion appears to be of a markedly chronic character.

As to the nature of these ulcers, the possibility of parasitic origin is excluded with certainty. Parasites or eggs of parasites have not been found on microscopy of the ulcers and thorough examination of the skin of these animals. Examination of the skin performed in the Royal Veterinary and Agricultural College has given the same negative result.

Conceivably the rater scanty supply of vitamin A might lower the resistance of the skin to infection. It has been found, however, that vitamin A given in large amounts has no prophylactic effect nor any therapeutic effect. Pellagra is out of the question, too; the ulcers do not in the least resemble a pellagrous dermatitis, and, besides, the animals have had a plentiful daily supply of vitamin B through the food (the yeast).

The lesion was further ascertained to appear also when the animals were placed in other quarters. Other cages were tried, too — wooden boxes with a good deal of sawdust in which the animals perhaps could clean themselves more easily — also with a negative result.

It was noticed, however, that similar efflorescences had never been observed in the breeding colony (which was quartered in another part of the town). This was further confirmed by thorough examination of the many hundred breeding animals: no skin

lesion was disclosed in any animal. Now 20 young animals (one month old) from the breeding colony were isolated in solitary cages but placed in the same rooms as the breeding animals; and in these 20 animals the skin affection made its appearance at the same time and to the same extent as in my own animals. This proves that there must be a connection between the appearance of the ulcers and the isolation of the animals.

Then 20 animals (males and females, $2^1/_2$—3 months old), presenting such excoriations were caged together in pairs, or in small groups (1 male + 3 females). Prior to their pairing, all the animals showed a moderate degree of the skin lesion (at that time there were no severe cases among my animals). After cohabitation for 1—3 months, the animals presented no longer any ulcer whatever; their skin was entirely intact. From this it is evident that cohabitation has a curative effect on the skin lesion.

Probably the healing of the ulcers is due to an increased resistance induced through the normal function of the sexual glands.

AGDUHR's investigations have shown that the absence of a normal sexual life in the small rodents is of considerable biological consequence, producing parenchymatous changes in the endocrine organs and being associated with a lowered resistance to many noxious factors. AGDUHR's experiments suggest very strongly that the increased resistance to toxic substances presented by animals with a normal sexual life is due to an increased secretion of gonadotrophie and sexual hormones, partly because the frequency of mitosis is considerably higher in the respective glands in paired animals than in unpaired, partly because administration of sexual hormones (estrin or testestrin) to castrated animals (rats, mice) and anterior pituitary gonadotrophin (antex, Leo) to animals living separated from the other sex, resulted in an increase in the resistance corresponding to the one that appeared when the animals had access to a normal sexual life.

Probably the development of the ulcers is then to be explained as follows: In those areas of the skin which the animals cannot reach with the snout (head, neck and shoulder regions, *i. e.*, the sites of predilection for the lesion) they clean themselves and scratch by means of their hind feet which takes place under considerable muscular exertion. During this procedure the long and sharp claws will often produce some small superficial scratches implying a great possibility of infection, as the claws of an animal are never quite clean even when the animal is raised under the

most hygienic conditions. In unpaired animals the scratching lesions may become the site of infectious processes because sexual abstinence lowers the resistance of the organism. A curative effect asserts itself during cohabitation with the other sex because the performance of the normal sexual functions gradually increases the natural resistance of the animal.

Even though this explanation probably is correct, it cannot be excluded that the healing of the ulcers under cohabitation takes places simply because the animals are licking each others' ulcers which thus heal up gradually under the influence of the bactericidal substances in the saliva. This explanation is suggested by the fact that the ulcers are found chiefly in those skin regions which the animal is not able itself to reach with its mouth. On the other hand, as a rule ulcers are also found outside these skin regions; indeed, one of the sites of choice for the lesion is the skin at the corners of the mouth, which the animals can easily reach with the tongue. The animals clean their face many times a day with their forepaws which they wet with saliva; yet small ulcers are commonly found in the skin of the face. Besides, it does not seem very likely that an animal may be able with its snout to reach in between the stiff, close-set, and sensitive vibrissæ of another animal, and punctate excoriations are often found just between the vibrissæ.

It is hard to say why the males in my experiments were attacked more often and more severely by the inflammatory skin lesion than were the females; AGDUHR observed a similar sex difference in this studies on the resistance. There is a tradition to the effect that, in general, large robust individuals of a given species are more susceptible to disease than those of the small wiry type, and that in illness the prognosis is less favorable. This may or may not be true, but the fact is that the relative weight of the organs — and thus of the reserves of the organism — is distinctly greater for small than for large individuals. In the rat the males are considerably larger than the females and the relative weight of the organs which take part in the defensive mechanism of the organism (lymph glands, spleen and liver) in the males is 15—30 % lower than in the females at the age when the infection sets in.

It may be that inhibition of such a primitive function as the normal sexual life lowers the resistance in man, too. AGDUHR has advocated such a view, pointing out, among other things, that it has been established statistically that married persons

live longer than single. For various reasons, such an argument is only of questionable value. On the other hand, the sexual function is biologically such a primitive and primordial manifestation of life that it will be reasonable a priori to expect a fundamental harmony between all the mammalian species in this respect.

As to the lifetime of the unpaired rats it may be mentioned that they generally died suddenly of some infection when they reached an age of $1^1/_2$—2 years; senile changes as reduction in weight, general atrophy of organs and tissue were never observed in my animals. The highest age ascertained in my material has been 28 months. This is a strikingly short lifetime in comparison with the statements (DONALDSON 1924, SLONAKER 1912 a. o.) made in the literature concerning the normal life cycle of the rat — all to this effect, that the lifetime of the rat must be set at 3—4 years. Possibly the shorter lifetime in my material is due to the lowered resistance brought about by the preclusion of a normal sexual life.

On the other hand, it is also conceivable that the short lifetime is characteristic of the rat strain I have employed — a question that cannot be settled at present, as the animals in the breeding colony are employed only up to the age of 1—$1^1/_2$ years, because their fertility diminishes after this age.

In the literature I have been able to find only one paper that may serve to throw some light on the relation between lifetime and sexual function — a paper by KOBOZIEFF (1931). In a fairly large material of mice (833 animals) he found 4.12 % of the breeding males to reach an age between $2^3/_4$ and 3 years, while only 0.22 % of the unpaired males reached this age. Of unpaired females, 0.75 % reached the same age, while none of the breeding females lived to be that old — a difference which KOBOZIEFF attributes to wear on the female organism owing to the many pregnancies, parturitions and suckling of the large litters.

Summary.

1. In albino rats living in sexual abstinence the appearance of infected scratches is a common lesion.

2. This affection is not seen in rats living a normal sexual life.

3. The affection is cured when the animals are given the chance of a normal sexual life.

4. The lifetime for unpaired rats is found to be considerably lower than the normal lifetime of the rat as given in numerous reports in the literature.

5. It is reasonable, therefore, to assume that inhibition of the normal sexual function lowers the resistance of the animals to infection, resulting in a shorter lifetime.

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Der Einfluss der Mandelsäure auf die Sauerstoffaufnahme und Ammoniakbildung im Nieren- und Lebergewebe.

Von

BIRGER HERNER.

(Eingereicht am 4. April 1942.)

Einleitung.

Im Jahre 1935 lancierte ROSENHEIM Ammoniumamygdalat als Azidose erzeugendes Mittel in die Zystopyelitistherapie. Die Mandelsäure (im folgenden abgekürzt Mds) ist seither sehr viel verwendet worden, besonders in Gestalt von Kalziumamygdalat und die reichhaltige Literatur hierüber umfasst gegenwärtig über 150 Arbeiten. Diese befassen sich hauptsächlich mit dem Einfluss der Mds auf den Säuregrad des Harns und der hiervon abhängigen Wirkung auf die Bakterienflora. Auch einige Untersuchungen über die Giftigkeit sehr grosser Dosen von Mds für Versuchstiere liegen vor; u. a. von SCHOVANEC, STOLZ und ZADINA (1939). Beobachtungen über die Bedeutung der Mds für das Säure-Basenverhältnis im Organismus mittels Untersuchung der Alkalireserve des Blutes beziehungsweise von dessen aktuellem Kohlensäuregehalt kenne ich bloss von GISSELSSON (1940), der die Wirkung einer dreitägigen Behandlung mit Kalzium- bzw. Natriumamygdalat am Menschen untersucht hat.

Es liegen indessen Beobachtungen vor, die darauf deuten, dass eine längerdauernde Behandlung mit Mds ernstlich in die Säure-Basenregulierung des Körpers eingreift. So veröffentlichte NIELSEN (1941) einen Fall, in dem langdauernde Azidosentherapie mit u. a. Mds zu einer hochgradigen Entkalkung des Skelettes führte. ODIN beobachtete in einem Fall von Zystopyelitis am Sahlgren-

schen Krankenhaus in Gotenburg gemäss persönlicher Mitteilung eine erstaunlich geringe Ammoniakproduktion bei langdauernder, intensiver Mds-Behandlung. Es besteht also Anlass zu dem Verdacht, die Ammoniakabwehr sei bei Mds-Azidose nicht wirksam genug, und der Organismus müsse daher andere Basen mobilisieren.

Als Ursache dieser ungenügenden Ammoniakabwehr lässt sich eine Vergiftung der desaminierenden Enzyme im Organismus denken. Mds würde also die Enzyme vergiften, die unter normalen Verhältnissen die Aminosäuren an dem der COOH-Gruppe nächstgelegenen, also an dem in α-Stellung zu ihr befindlichen, Kohleatom angreifen. Hier fängt gewöhnlich der Abbau der Aminosäuren mit einer Desaminierung an. Aus Versuchen von LEHMANN (1938) über die Fähigkeit gewisser Hefezellen Milchsäure zu oxydieren, ging hervor, dass Mds eine wesentliche Hemmung ausübte, und da erwiesen ist, dass die Oxydation der Milchsäure beim α-Kohleatom beginnt, könnte dies also die Annahme stützen, dass Mds eine Tendenz zeigt enzymatische Prozesse, die an dieser Stelle angreifen, zu stören. Die Erklärung hierfür kann darin zu sehen sein, dass die Mds im Organismus offenbar selbst zuerst beim α-Kohleatom abgebaut wird und sie deshalb die hier angreifenden Enzyme belegt.

Betreffend das Verhalten der Mds im Organismus liegen Versuche von SCHOLZ (1937) und MONTENBRUCK (1940) vor, die beide ihre Versuchspersonen Mds einnehmen liessen und dann den Harn untersuchten. Sie fanden darin ungefähr 70 % der Mds unverändert wieder, etwa 15 % als Phenylglyoxylsäure (Benzoylameisensäure) sowie bis zu ungefähr 13 % Benzoësäure. MONTENBRUCK konnte überdies eine geringere Menge Phenylaminoessigsäure nachweisen.

Die Formeln dieser Stoffe haben folgendes Aussehen:

$C_6H_5 \cdot CH(OH) \cdot COOH$ Mandelsäure

$C_6H_5 \cdot CO \cdot COOH$ Phenylglyoxylsäure

$C_6H_5 \cdot COOH$ Benzoësäure

$C_6H_5 \cdot CH(NH_2) \cdot COOH$ Phenylaminoessigsäure

Es zeigt sich, dass die obigen Umsetzungsprodukte der Mds gerade den Abbauprodukten entsprechen, die man vom enzymchemischen Standpunkt aus für sie erwarten möchte. Ebenso wie die Milchsäure, mit der sie die CH(OH)·COOH-Gruppe gemeinsam hat, wird Mds zunächst am α-Kohleatom oxidiert und dann in analoger Weise weiter abgebaut.

Die entsprechenden Umsetzungsprodukte der Milchsäure sind die folgenden:

$CH_3 \cdot CH(OH) \cdot COOH$	$CH_3 \cdot CO \cdot COOH$	$CH_3 \cdot COOH$
Milchsäure	Brenztraubensäure	Essigsäure

Wir sehen also, dass vieles dafür spricht, dass der Abbau von Mds am α-Kohleatom beginnt. Die von MONTENBRUCK nachgewiesene Phenylaminoessigsäure bildet sich wahrscheinlich in der Niere durch sogenannte Umaminierung, indem eine NH_2-Gruppe aufgenommen wird.

Aus den oben referierten Versuchen von SCHOLZ und MONTENBRUCK geht hervor, dass Mds im Organismus bis zu einem gewissen Grad umgesetzt wird. Es schien daher von Interesse, in Gewebeatmungsversuchen, vor allem mit Niere und Leber, zu untersuchen, welche Organe imstande sind Mds zu oxydieren. Durch diese Versuche muss weiterhin die etwaige toxische Wirkung der Mds sowohl auf die Desaminierung als auch auf die Oxydation der Aminosäuren erkennbar werden. In erster Linie ist eine Untersuchung des Sauerstoffverbrauchs geeignet, Aufklärung hierüber zu verschaffen. Der Abbau der Aminosäuren im Organismus wird in der Regel durch eine Desaminierung eingeleitet, die eine Ketosäure ergibt, welche sodann oxydiert und dekarboxyliert wird. Unveränderter Sauerstoffverbrauch vor und nach Zusatz von Mds im Versuch muss demnach für ungestörte Desaminierung wie auch für ungestörte Oxydation sprechen. Erhält man dagegen einen verminderten Sauerstoffverbrauch nach Zusatz von Mds, so kann dies auf einer Störung des einen der beiden Vorgänge oder gleichzeitig beider beruhen. Eine nähere Lokalisation muss auf dem Wege der Analyse einerseits der Desaminierungs-, anderseits der Oxydationsprozesse möglich sein.

Diese Fragestellungen wurden nun in Versuchen studiert, die im folgenden beschrieben werden. Die angeführten Versuche sind typische Beispiele, deren Ergebnisse durch eine Anzahl gleichartiger Versuche bestätigt wurden.

I. Versuche über die Einwirkung von Mandelsäure auf den Sauerstoffverbrauch in verschiedenen Geweben.

Versuchstechnik. Als Versuchsobjekt wurden Organe von weissen Ratten verwendet. Die Tiere wurden dekapitiert, worauf in

unmittelbarer Folge die Organe entnommen und in einer in Eiswasser stehenden Schale gekühlt wurden. Für die Versuche wurden Gewebs*schnitte*, Gewebs*brei* und *Extrakt* von Gewebsbrei verwendet. Die *Schnitte* wurden in gewöhnlicher Weise mit dem Rasiermesser angefertigt. Sie wurden in eisgekühlte KREBS-RINGER-Lösung (1932) gelegt und vor der Wägung durch Abtrocknen auf Filtrierpapier von freier Flüssigkeit befreit. Der *Brei* wurde durch Feinschneiden mit der Schere bis zu homogener, zähflüssiger Konsistenz hergestellt. Die Präparation erfolgte auf eisgekühlter Unterlage. Das Gewebsbrei*extrakt* ist im grossen und ganzen nach BERNHEIM (1934) bereitet worden: Die Organe wurden feingeschnitten, wie eben angegeben; in den Versuchen mit Leber- und Muskelextrakt wurden gleiche Mengen von Organbrei und KREBS-RINGER-Lösung gemischt; in den Nierenextraktversuchen wurde halb soviel Brei verwendet. Die Mischung wurde einige Minuten zusammen mit feingemahlenem Glas im Achatmörser gerieben und dann durch eine doppelte Lage Gaze filtriert. Bis zur Verwendung in den Versuchen wurde das Extrakt bei 0° C aufbewahrt.

Die Versuche wurden mit *Warburg-Respirationsgefässen* von ca 16 ml Rauminhalt und mit Luft als Sauerstoffquelle ausgeführt. Die Flüssigkeitsmenge in den Gefässen, einschliesslich der Versuchsgewebe, war 3.00 ml. In den Einsätzen wurde 0.20 ml 5%-ige Kalilauge verwendet. Der Zusatz verschiedener Stoffe im Lauf des Versuchs erfolgte von der Birne aus, die sich an der Seite der Gefässe befindet. Die Wassertemperatur im Brutschrank war 37° C. Um Temperatur- und Gasgleichgewicht in den Gefässen zu erreichen, wurden diese im Brutschrank 15 min geschüttelt, ehe die Hähne geschlossen wurden und die Ablesung anfing. Die Frequenz beim Schütteln war ungefähr 120 Schläge in der Minute und die Amplitude ca 4 cm.

Die zu den Versuchen verwendete *Mds* (SCHERING-KAHLBAUMS »Acidum amygdalicum für wissenschaftliche Zwecke«) wurde mit Natronlauge neutralisiert und somit in Gestalt von Natriumamygdalat verwendet. Kalziumamygdalat, das, wie erwähnt, in klinischen Versuchen verwendet worden ist, schien für Gewebsversuche ungeeignet, weil Kalziumjonen in stärkerer Konzentration störend auf die Oxydation der Zellen wirken. Die verwendeten *Aminosäuren* waren Mercks dl-Alanin und dl-Prolin von Heyl und Co, beides Analysenpräparate.

1. Der Sauerstoffverbrauch im Nierengewebe mit und ohne Zusatz von Mandelsäure.

N i e r e n s c h n i t t (Versuch 1 und Figur 1).

Reaktionsgemisch: 2.65 ml KREBS-RINGER-Lösung (von nun an K-R-Lös.) + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat in den Birnen. Nierenschnitt 0.05 g.

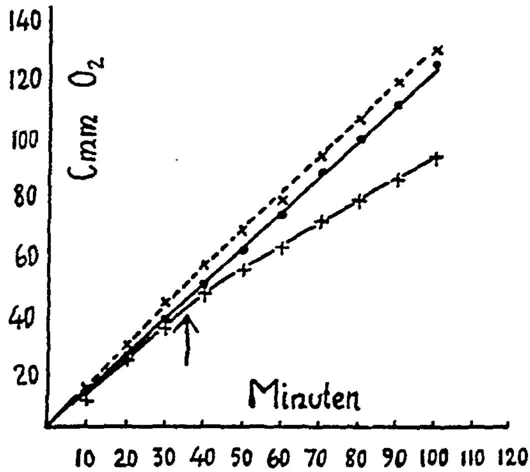


Fig. 1 zeigt die Spontanatmung sowie den Einfluss von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenschnitt.

Der Pfeil gibt an wenn Mds zugesetzt wurde.

Atmung ohne Zusatz von Mds . . . . ×------×
bei Zusatz von 0.01 mol Mds . . . . •———•
bei Zusatz von 0.1 mol Mds . . . . +———+

In diesem wie auch in allen folgenden Versuchen wurde ein Versuch über die Spontanatmung gemacht, wobei Mds durch dieselbe Menge K-R-Lös. ersetzt wurde.

Aus dem Versuch geht hervor, dass der Sauerstoffverbrauch bei einer Mds-Konzentration von 0.1 mol im Reaktionsgemisch erheblich sinkt, wogegen eine Konzentration von 0.01 mol keine sichere Abweichung von der Spontanatmung ergibt. Ebensowenig ergaben Versuche mit 0.001 mol eine klare Abweichung.

N i e r e n b r e i (Versuch 2 und Figur 2).

Reaktionsgemisch: 2.63 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat in den Birnen. Nierenbrei 0.07 g. Technik im übrigen wie in Versuch 1.

Das Versuchsergebnis stimmt mit dem beim Nierenschnittversuch erhaltenen überein.

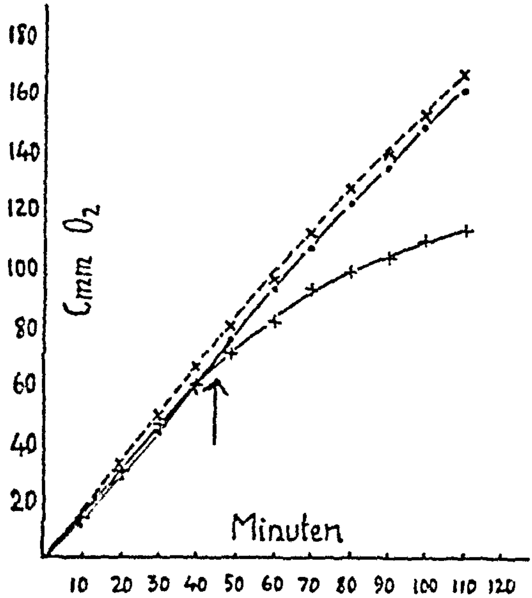


Fig. 2 zeigt die Spontanatmung sowie den Einfluss von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenbrei.

Der Pfeil gibt an wenn Mds zugesetzt wurde.

Atmung ohne Zusatz von Mds . . . . ×------×
bei Zusatz von 0.01 mol Mds . . . . ·——————·
bei Zusatz von 0.1 mol Mds . . . . +——————+

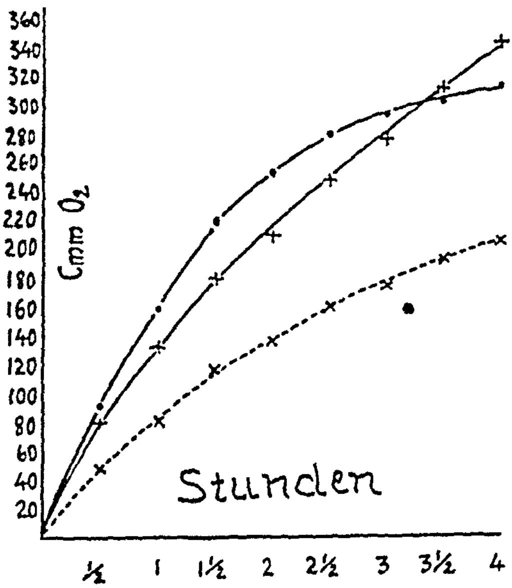


Fig. 3 zeigt die Spontanatmung sowie den Einfluss von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenextrakt.

Atmung ohne Zusatz von Mds . . . . ×------×
bei Zusatz von 0.01 mol Mds . . . . ·——————·
bei Zusatz von 0.1 m l Mds . . . . +——————+

N i e r e n e x t r a k t (Versuch 3 und Figur 3).

Reaktionsgemisch: 1.70 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat in den Birnen. Nierenextrakt 1.00 ml.

In diesem Versuch zeigt sich eine beträchtliche Mehrung des Sauerstoffverbrauchs bei einer Mds-Konzentration von 0.1 und 0.01 mol im Reaktionsgemisch. Die schwächere Mds wirkt anfangs stärker anregend als die stärkere, aber nach 2 std ist ihre Wirkung vorbei, und der Sauerstoffverbrauch läuft fernerhin parallel mit dem im Spontanatmungsversuch. Die stimulierende Wirkung der stärkeren Mds-Konzentration dagegen bleibt über die 4 std der Versuchsdauer erhalten. Die Kurven in Fig. 3 haben einen mehr bogenförmigen Verlauf als die in Fig. 1 und 2 bei Schnitt und Brei. Dies deutet darauf, dass im Lauf des Versuchs der Stoffwechsel im Nierenextrakt kontinuierlich abnimmt, was seine Erklärung darin finden dürfte, dass die Nährstoffe der Zellen, die im Extrakt ja verdünnt vorliegen, aufgebraucht werden und vielleicht auch darin, dass im Laufe des Versuchs eine Enzymzerstörung stattfinden könnte. Im Extrakt sind ja auch die für einen anhaltenden, ungestörten Stoffwechsel nötigen Enzyme nicht in der selben Konzentration und nicht im selben gegenseitigen Verhältnis vorhanden wie in Schnitt und Brei.

Die Ergebnisse dieser drei Versuche können folgendermassen zusammengefasst werden: In Versuchen mit *Nierenschnitt* und *Nierenbrei* hemmt eine Mds-Konzentration von 0.1 mol die Gewebeatmung beträchtlich, während eine Konzentration von 0.01 mol nicht mit Sicherheit eine hemmende Wirkung hat. Im *Nierenextrakt*versuch stimuliert eine Mds-Konzentration von 0.01 mol die Gewebeatmung stark, während eine Konzentration von 0.1 mol sowohl eine hemmende als auch eine stimulierende Wirkung auszuüben scheint. Die Bedeutung dieser Ergebnisse soll später im Zusammenhang mit den übrigen Versuchsergebnissen besprochen werden.

2. Der Sauerstoffverbrauch im Nierengewebe bei Anwesenheit von Alanin und Prolin sowie von Mandelsäure in verschiedener Konzentration.

N i e r e n s c h n i t t + A l a n i n (Versuch 4 und Figur 4).

Reaktionsgemisch: 2.35 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat + 0.30 ml 0.1 mol Alaninlösung (= 2.67 mg) in den Birnen. Nierenschnitt 0.05 g.

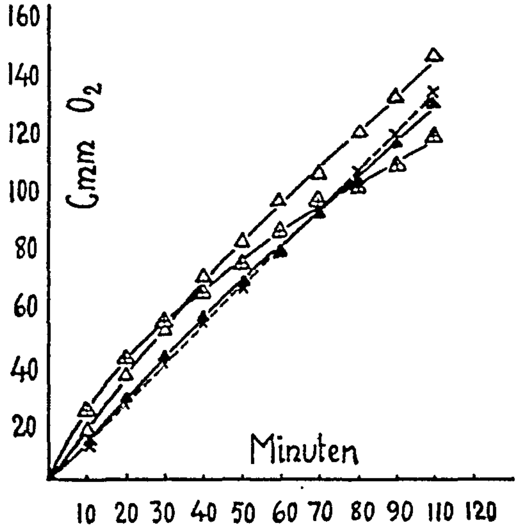


Fig. 4 zeigt die Spontanatmung sowie den Einfluss von Alanin ohne und bei Zusatz von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenschnitt.

Atmung ohne Zusatz von Alanin und Mds . . . . ×------×
bei Zusatz von 0.01 mol Alanin . . . . △——△
bei Zusatz von 0.01 mol Alanin + 0.01 mol Mds ▲——▲
bei Zusatz von 0.01 mol Alanin + 0.1 mol Mds ⊼——⊼

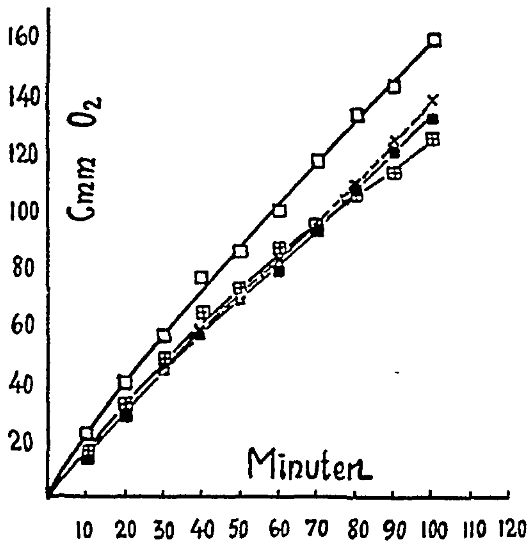


Fig. 5 zeigt die Spontanatmung sowie den Einfluss von Prolin ohne und bei Zusatz von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenschnitt.

Atmung ohne Zusatz von Prolin und Mds . . . . ×------×
bei Zusatavon 0.01 mol Prolin . . . . □——□
bei Zusatz von 0.01 mol Prolin + 0.01 mol Mds ■——■
bei Zusatz von 0.01 mol Prolin + 0.1 mol Mds ⊞——⊞

N i e r e n s c h n i t t + P r o l i n (Versuch 5 und Figur 5). Die selben Versuchsanordnungen, die Alaninlösung jedoch durch 0.30 ml 0.1 mol Prolinlösung (= 3.45 mg) ersetzt.

Die Versuchsergebnisse zeigen, dass sowohl Alanin als auch Prolin die Tendenz haben, den Sauerstoffverbrauch zu stimulieren. Gleichzeitiger Zusatz von Mds in einer Konzentration von 0.01 mol scheint den Sauerstoffverbrauch etwas zu mindern. Das Ergebnis ist jedoch nicht völlig eindeutig. Bei einer Mds-Konzentration von 0.1 mol lässt sich eine weitere Hemmung des Sauerstoffverbrauchs feststellen, die zwar auch nicht besonders stark ausgeprägt ist, immerhin aber deutlich tiefer liegt als bei den Versuchen mit ausschliesslich Aminosäurezusatz.

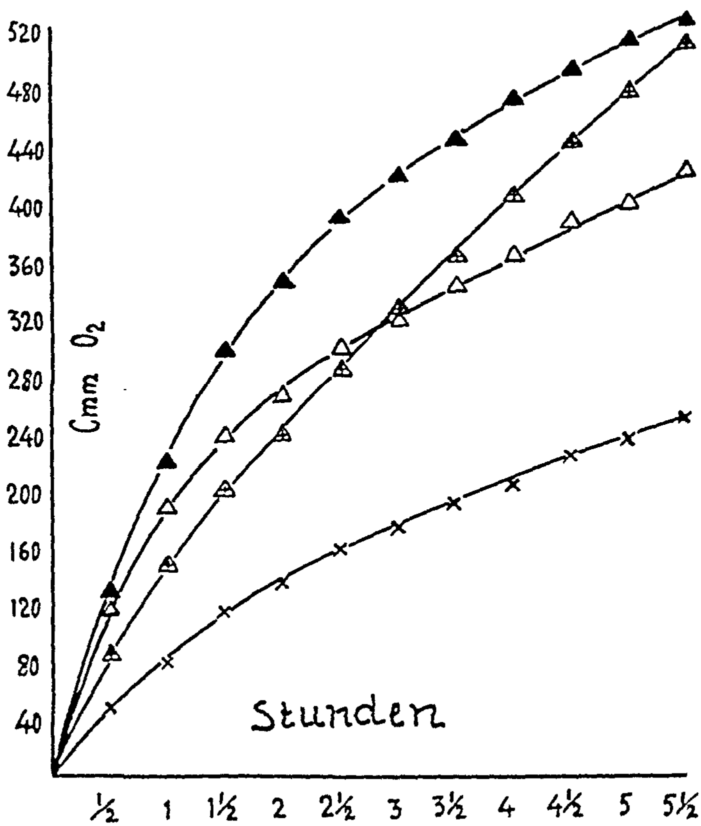


Fig. 6 zeigt die Spontanatmung sowie den Einfluss von Alanin ohne und bei Zusatz von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenextrakt.

Atmung ohne Zusatz von Alanin und Mds . . . . ×——×
bei Zusatz von 0.01 mol Alanin . . . . △——△
bei Zusatz von 0.01 mol Alanin + 0.01 mol Mds ▲——▲
bei Zusatz von 0.01 mol Alanin + 0.1 mol Mds ⨺——⨺

Nierenextrakt + Alanin (Versuch 6 und Figur 6). Reaktionsgemisch: 1.40 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat + 0.30 ml 0.1 mol Alaninlösung in den Birnen. Nierenextrakt 1.00 ml.

Nierenextrakt + Prolin (Versuch 7 und Figur 7).

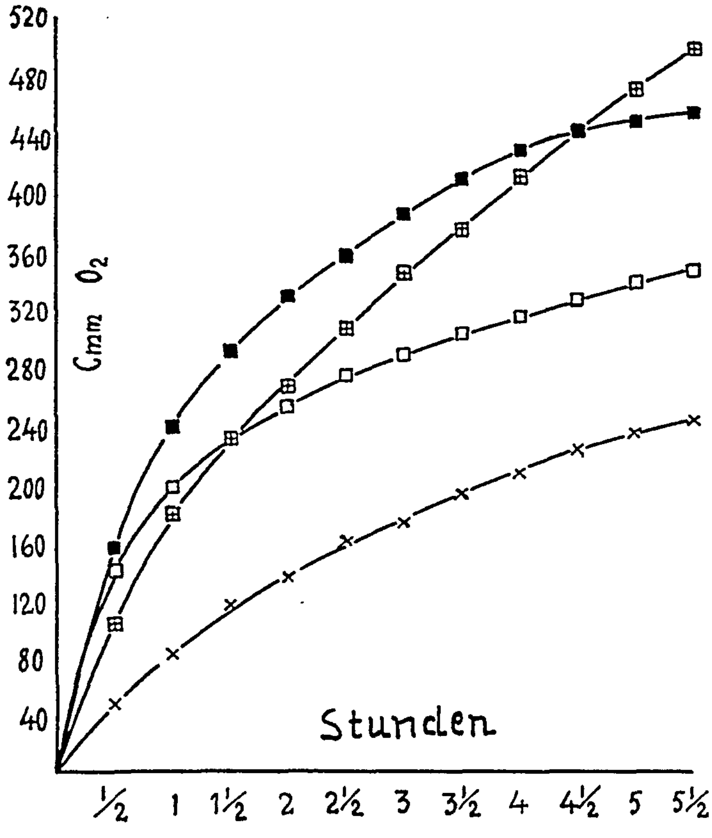


Fig. 7 zeigt die Spontanatmung sowie den Einfluss von Prolin ohne und bei Zusatz von Mandelsäure verschiedener Konzentration auf den Sauerstoffverbrauch im Nierenextrakt.

Atmung ohne Zusatz von Prolin und Mds . . . . ×———×
bei Zusatz von 0.01 mol Prolin . . . . □———□
bei Zusatz von 0.01 mol Prolin + 0.01 mol Mds ■———■
bei Zusatz von 0.01 mol Prolin + 0.1 mol Mds ⊞———⊞

Die selben Versuchsanordnungen, Alanin jedoch ersetzt durch Prolin.

Aus den Versuchen geht hervor, dass Alanin und Prolin je für sich den Sauerstoffverbrauch wesentlich stimulieren. Bei Ablesung nach 4 std zeigt sich eine Steigerung von ungefähr 80 bzw. 50 %. Gleichzeitiger Zusatz von Mds in einer Konzentration von 0.01 mol

zum Reaktionsgemisch stimuliert den Sauerstoffverbrauch noch mehr. Dieser wird durch die angegebene Mds-Konzentration ungefähr ebenso stark gesteigert wie in Versuch 3, wo der Mds-Effekt für sich studiert wurde. Die beiden Stoffe scheinen also einen additiven Effekt zu haben. Eine Mds-Konzentration von 0.1 mol scheint anfangs eine recht deutlich hemmende Wirkung auszuüben, verglichen mit Versuchen mit 0.01 mol Mds. Verglichen mit ausschliesslich Aminosäurezusatz zeigen Versuche mit Alanin und Prolin + 0.1 mol Mds nach 3 bzw. 1.5 std einen stärkeren Sauerstoffverbrauch. (Die Kurven kreuzen sich). Dies deutet darauf, dass Mds auch in dieser Konzentration dazu beitrug, den Sauerstoffverbrauch anzuregen, aber zu diesem Zeitpunkt in geringerem Masse als in Versuchen mit 0.01 mol Mds.

3. Der Sauerstoffverbrauch im Lebergewebe mit und ohne Zusatz von Mandelsäure.

Die Resultate der Versuche mit Lebergewebe stimmen der Hauptsache nach mit den Nierengewebsversuchen überein; sie wurden daher nicht graphisch, sondern tabellarisch (Tab. 1) wiedergegeben.

Leberschnitt (Versuch 8).
Reaktionsgemisch: 2.60 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat in den Birnen. Leberschnitt 0.10 g.

Leberextrakt (Versuch 9).
Reaktionsgemisch: 1.70 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat in den Birnen. Leberextrakt 1.00 ml.
Die Versuche mit Leberschnitt und -Extrakt ergaben ähnliche Resultate wie gleichartige Versuche mit Niere.

Leberbrei.
In Versuchen mit Leberbrei als Substrat wurden keine befriedigenden Ergebnisse erzielt, da der spontane Sauerstoffverbrauch des Gewebes allzurasch reduziert wurde.

4. Der Sauerstoffverbrauch im Lebergewebe bei Anwesenheit von Alanin und Prolin sowie Mandelsäure in verschiedener Konzentration.

Leberschnitt + Alanin (Versuch 10).
Reaktionsgemisch: 2.35 ml K-R-Lös. + 0.30 ml 1 mol bzw.

0.1 mol Natriumamygdalat + 0.30 ml 0.1 mol Alaninlösung in den Birnen. Leberschnitt 0.05 g.

Leberschnitt + Prolin (Versuch 11).

Die selben Versuchsanordnungen, Alanin jedoch durch Prolin ersetzt.

Auch bei diesen Versuchen stimmen die Ergebnisse im Prinzip mit denen entsprechender Nierenversuche überein, mit einer klaren Hemmung des Sauerstoffverbrauchs in Versuchen mit der stärkeren Mds-Konzentration.

Leberextrakt + Alanin (Versuch 12).

Reaktionsgemisch: 1.40 ml K-R-Lös. + 0.30 ml 1 mol bzw. 0.1 mol Natriumamygdalat + 0.30 ml 0.1 mol Alaninlösung in den Birnen. Leberextrakt 1.00 ml.

Leberextrakt + Prolin (Versuch 13).

Die selben Versuchsanordnungen, Alanin jedoch durch Prolin ersetzt.

Auch aus diesen Versuchen geht, wie aus entsprechenden Nierenversuchen, die additive Wirkung der Mds und der Aminosäuren auf die Sauerstoffaufnahme hervor, sowie auch die stärker anregende Wirkung der schwächeren Mds in den ersten Stunden.

5. Der Sauerstoffverbrauch im Muskelgewebe mit und ohne Alanin und Mandelsäure in verschiedener Konzentration.

In Versuchen mit einerseits Brei, anderseits Extrakt wurde bei gleicher Versuchsanordnung wie der für Niere und Leber beschriebenen sowohl bei Spontanatmungsversuchen als auch bei Versuchen mit Alanin und Mds-Zusatz ein sehr geringer Sauerstoffverbrauch festgestellt. Es liessen sich deshalb aus dem Einfluss dieser Stoffe auf den Sauerstoffverbrauch keine sicheren Schlüsse ziehen.

6. Der Sauerstoffverbrauch im Hirngewebe mit und ohne Mandelsäure in verschiedener Konzentration.

In Versuchen mit Hirnextrakt — angeordnet wie die zuvor beschriebenen Versuche — ergab sich bei Zusatz von 0.1 mol Mds zum Reaktionsgemisch eine kräftige Hemmung. Der Sauerstoffverbrauch in Versuchen ohne Mds war nach 4 std 209 mm^3, in Versuchen mit 0.1 mol Mds 128 mm^3.

Tabelle 1.

Der Sauerstoffverbrauch im Lebergewebe mit und ohne Mandelsäure in Konzentration von 0.1 und 0.01 mol sowie mit und ohne Alanin bzw. Prolin.

Substrat	Sauerstoffverbrauch in cmm nach				
	0.5 std	1 std	2 std	3 std	4 std
8. *Leberschnitt:*			(1.5 std)		
Spontanatmung (sp)	43	80	122	—	—
» + 0.01-m Mds	35	67	101	—	—
» + 0.1-m Mds	20	28	42	—	—
9. *Leberextrakt:*			(2 std)		
sp	59	78	106	133	154
sp + 0.01-m Mds	64	90	130	164	193
sp + 0.1-m Mds	57	87	140	192	240
10. *Leberschnitt + Alanin:*					
sp	22	36	79	115	145
sp + Alanin	24	45	89	129	163
sp + Alanin + 0.01-m Mds	24	46	94	138	174
sp + Alanin + 0.1-m Mds	29	45	76	99	105
11. *Leberschnitt + Prolin:*					
sp	21	36	79	115	154
sp + Prolin	26	40	106	154	195
sp + Prolin + 0.01-m Mds	25	43	90	132	171
sp + Prolin + 0.1-m Mds	25	41	84	117	145
12. *Leberextrakt + Alanin:*					
sp	40	56	78	90	102
sp + Alanin	53	80	113	131	149
sp + Alanin + 0.01-m Mds	60	101	147	182	210
sp + Alanin + 0.1-m Mds	56	77	125	163	197
13. *Leberextrakt + Prolin:*					
sp	32	80	134	160	190
sp + Prolin	65	180	252	293	320
sp + Prolin + 0.01-m Mds	65	181	280	352	370
sp + Prolin + 0,1-m Mds	40	122	204	294	320

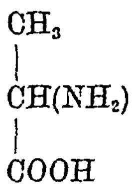
Diskussion.

Aus den Versuchen ohne Zusatz von Aminosäuren geht hervor, dass Mds in einer Konzentration von 0.1 mol die spontane Oxydation im *Nierenbrei, Nierenschnitt und Leberschnitt* wesentlich hemmt. In versuchen mit *Extrakt von Niere und Leber* lässt sich dagegen sowohl bei einer Mds-Konzentration von 0.1 als auch bei einer solchen von 0.1 mol eine Mehrung der Oxydation beobachten. Bei der stärkeren Konzentration tritt allerdings, verglichen mit der schwächeren, eine anfängliche Hemmung der Oxydation auf, aber dies ist vielleicht nur einer Salzwirkung zuzuschreiben. Die Mehrung des Sauerstoffverbrauchs, die bei den *Extrakt*versuchen auftritt, dürfte auf einer Verbrennung der zugesetzten Mds beruhen. Der Anlass dazu, dass diese Verbrennung sich nur in den *Extrakt*versuchen geltend macht, nicht aber in den Versuchen mit *Schnitt* und *Brei*, kann möglicherweise darin zu sehen sein, dass diese Gewebe einen weniger gestörten Zugang zu Nährstoffen haben. In den *Extrakten* liegen nämlich, wie erwähnt, veränderte Verhältnisse vor, insofern als die natürlichen Nährstoffe verdünnt sind, weshalb die Mds hier in höherem Masse als Ersatznährstoff auftreten kann. Inwieweit die Mds gleichzeitig hemmend auf die Oxydation anderer Stoffe im Gewebe wirkt, lässt sich aus diesen Versuchen nicht ersehen, da der erhöhte Sauerstoffverbrauch durch die Oxydation der Mds vielleicht eine Hemmung in anderen Oxydationsprozessen überdeckt. Der Sauerstoffverbrauch scheint von der Mds in Nierenversuchen stärker als in Leberversuchen angeregt zu werden, was dafür spricht, dass die Niere Mds leichter verbrennt.

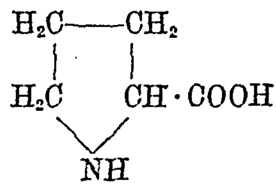
Aus den Versuchen mit *Nierenextrakt* (3, 6, 7) scheint hervorzugehen, dass die Mehrung des Sauerstoffverbrauchs, die durch 0.01 mol Mds im Reaktionsgemisch hervorgerufen wird, sich bei ungefähr 100 mm^3 Mehraufnahme stabilisiert. Zum Abbau der zugefügten Menge Mds (0.03 millimol) bis auf das als nächstes angenommene Abbauprodukt, Phenylglyoxylsäure, sind für die Oxydation ungefähr 325 mm^3 Sauerstoff erforderlich. Unter der Voraussetzung, dass die Oxydation nicht weiter schreitet als bis zur Phenylglyoxylsäure, sind also etwa 30 % der zugesetzten Menge Mds oxydiert worden.

Es scheinen also vor allem Niere und Leber zu sein, die die nach früheren Versuchen von SCHOLZ und MONTENBRUCK im Organismus stattfindende Verbrennung von Mandelsäure besorgen.

Aus den Versuchen mit *Schnitten* und *Brei* von Niere und Leber ging, wie bereits gesagt, hervor, dass Mds eine Oxydationshemmung verursachte. Es ist indessen nicht möglich, an Hand dieser Versuche allein mit Sicherheit zu bestimmen, welche Stoffe es sind, deren Oxydation durch Mds gehemmt wird. In der Einleitung wurde jedoch hervorgehoben, dass Anlass zu der Vermutung bestehe, die Mds hemme den Abbau der Aminosäuren. Es ist somit denkbar, dass die Minderung der Sauerstoffaufnahme in den Versuchen wenigstens teilweise durch eine Blockierung des Abbaus der Aminosäuren verursacht wird. Um dies zu untersuchen sind daher Versuche mit Zusatz von Aminosäuren angestellt worden. Hierzu wurden sowohl Alanin als auch Prolin gewählt, weil diese beiden Aminosäuren im Organismus auf verschiedene Weise abgebaut werden (BERNHEIM 1934) und deshalb vielleicht sich ergänzende Aufschlüsse darüber geben können, wo die Hemmung seitens der Mds einsetzt. Alanin wird nämlich zuerst am α-Kohleatom abgebaut, fürs erste in Gestalt einer Desaminierung und nachher in der einer Oxydation, während Prolin nicht desaminiert, sondern nur oxydiert wird, nach BERNHEIM (1936) an der NH- und der daneben sitzenden CH_2-Gruppe.



Alanin



Prolin

Eine Hemmung der Sauerstoffaufnahme bei Alanin-Mds-Versuchen kann somit eine Blockierung der Desaminierung beim α-Kohleatom und eine dadurch ebenfalls blockierte Oxydation oder bloss das letztere bedeuten. Eine Hemmung der Sauerstoffaufnahme bei Prolin-Mds-Versuchen kann dagegen bedeuten, dass Mds die Oxydation auf andere Weise blockiert.

In den Versuchen mit Nieren- und Leber*schnitten* wird die Sauerstoffaufnahme durch Zusatz von sowohl Alanin als auch Prolin etwas gemehrt. Sie wird jedoch gemindert durch gleichzeitigen Zusatz von Mds, obwohl im Vergleich zur Spontanatmung weder Mds von 0.1 noch von 0.01 mol eine ausgesprochene Hemmung zustandebringt. Die Versuche sprechen aber dafür, dass Mds ihre Hemmung auf die Oxydation der Aminosäuren ausübt. Da die Hemmung ebensowohl in Alanin- als auch in Prolinversuchen

auftritt, spricht dies, wie eben angedeutet, dafür, dass Mds nicht ausschliesslich Prozesse, die am α-Kohleatom der Aminosäuren stattfinden, vergiftet.

In Versuchen mit sowohl Nieren- als auch Leber*extrakt* bei gleichzeitigem Aminosäure- und Mds-Zusatz entsteht eine kräftige, offenbar additive Mehrung des Sauerstoffverbrauchs. Dies macht es unmöglich, aus der Grösse des Sauerstoffverbrauchs Schlüsse auf das Vorliegen einer Hemmung hinsichtlich des Abbaus der zugesetzten Aminosäuren zu ziehen. Um hier den Dingen auf den Grund zu kommen, ist in den folgenden Versuchen das im Verlauf eines jeden gebildete Ammoniak bestimmt worden, da dieses, wie erwähnt, was Alanin betrifft, zuerst abgespalten wird, noch ehe eine Oxydation stattfinden kann. Ähnliche Versuche wurden auch mit Prolin ausgeführt.

II. Gewebsversuche über den Einfluss der Mandelsäure auf den Sauerstoffverbrauch sowie auf die Ammoniakbildung.

Die Sauerstoffversuche wurden in derselben Weise ausgeführt wie die zuvor beschriebenen Versuche mit Nierenbrei und -Extrakt sowie mit Leberschnitten und -Extrakt. Im Anschluss an diese Versuche wurden ausserdem *Ammoniakbestimmungen* vorgenommen.

Die Bestimmung wurde nach der Diffusionsmethode von CONWAY (1933) ausgeführt, wenn auch in etwas abgeänderter Form. Die von CONWAY verwendeten Diffusionskammern sind nämlich für diese Versuche nicht geräumig genug. Die Ammoniakbestimmung muss ja am ganzen Inhalt des Warburg-Gefässes vorgenommen werden, der aus 3 ml Analysenflüssigkeit und überdies 2 ml Spülwasser besteht. CONWAYS Kammern sind aber nur für höchstens 3 ml Analysenflüssigkeit berechnet. Es wurden deshalb Diffusionskammern aus einer grösseren Petrischale von 60 mm Durchmesser und 12 mm Tiefe sowie einer kleineren von 40 bzw. 7 mm angefertigt. Die beiden Schalen wurden mit einer dünnen Paraffinschicht zusammengeschmolzen, die kleine in der grossen, worauf die ganze Kammer inwendig paraffiniert wurde. Die Diffusionskammern wurden standardisiert für 5 ml Analysenflüssigkeit in der Aussenkammer, 5 ml 0.01-n Salzsäure in der Innenkammer sowie weiterhin 1 ml gesättigte Natriumkarbonatlösung in der Aussenkammer, dazu bestimmt, das Ammoniak frei-

zumachen. Um das von der Salzsäure gebundene Ammoniak zu bestimmen, wurden, je nach der Ammoniakkonzentration, 1—3 ml Säure nesslerisiert (Probe + 0.2 ml Einäscherungslösung + Wasser auf 7 ml + 3 ml Nesslers Reagens). Die Bestimmungen wurden in PULFRICHS Photometer mit Filter S 50 bei 1 cm Schichtdicke gemacht. Die erhaltenen Extinktionswerte wurden nach einem Diagramm, das nach bekannten Mengen Ammoniumsulfats hergestellt worden war, in entsprechende Milligramm Ammoniak verwandelt. Bei den Standardisierungsversuchen zeigte es sich, dass bei Zimmertemperatur eine Diffusionszeit von 4 std für quantitativen Austausch genügt. Die Fehler überstiegen hierbei niemals 4 %. Im allgemeinen war jedoch in den Versuchen die Diffusionszeit bedeutend länger, ungefähr 12—16 std.

Um zu kontrollieren wieviel von dem während des Sauerstoffversuches gebildeten Ammoniak vom Reaktionsgemisch bei pH 7.4 gebunden wird, wurden Kontrollversuche angestellt. Ein ml Ammoniumsulfatlösung mit entsprechender Ammoniakmenge wie in den Extrakten ersetzte diese in den Warburg-Gefässen. Die Gefässe wurden dann im Brutschrank 4 std geschüttelt. Unmittelbar zu Anfang des Versuchs sowie nach 2 und nach 4 std wurde 1 ml Flüssigkeit aufgenommen, nesslerisiert und analysiert. Die Ergebnisse zeigen, dass kein Ammoniakverlust entsteht, sondern dass alles Ammoniak gebunden in der Flüssigkeit bleibt. Bei einem der Versuche ergaben sich folgende Werte: 0.132; 0.123; 0.135 mg Ammoniak nach bzw. 0, 2 und 4 std.

Die *Reaktionsgemische* und Gewebemengen waren dieselben wie bei den entsprechenden, früher beschriebenen Versuchen.

Bei den verschiedenen Versuchsserien wurden vergleichende Ammoniakanalysen vorgenommen in Versuchen mit und ohne Zusatz von Mds in verschiedener Konzentration. Zu Beginn jeder Versuchsserie wurde nach der Temperatureinstellung eine Probe analysiert, um so einen Blindwert für die schon von vornherein im Reaktionsgemisch (im Gewebe) vorhandene Ammoniakmenge zu erhalten. Im allgemeinen liefen die Versuche schon 4 std, ehe die Ammoniakbestimmungen vorgenommen wurden; in einzelnen Versuchen wurde jedoch die Ammoniakbildung Stunde für Stunde untersucht. Es schien unzweckmässig, die Versuche über länger als 4 std auszudehnen, da sich dabei eine Bakterienwirkung geltend machen könnte.

In unterstehender Tabelle sind die verschiedenen Versuche zusammengestellt. Die Ergebnisse zeigen die Wirkung von 0.1 mol

Mds-Konzentration auf die Ammoniakbildung in verschiedenen Versuchen mit und ohne Alanin und Prolin. Aus Kontrollversuchen geht hervor, dass die Resultate innerhalb 10 % reproduzierbar sind.

Tabelle 2.

1	2	3	4	5	6	7	8	9	10
Versuchssubstrat	mg H_3N in Blindprobe	mg H_3N nach 4 std ohne Mds	mg H_3N nach 4 std mit 0.1-m Mds	mg gebildetes H_3N in Versuchen ohne Mds (Kol. 3 — Kol. 2)	mg gebildetes H_3N in Versuchen mit Mds (Kol. 4 — Kol. 2)	von Mds gehemmte H_3N-Bildung in Prozenten	O_2-Verbrauch in cmm nach 4 std ohne Mds	O_2-Verbrauch in cmm nach 4 std mit Mds	O_2-Verbrauchssteigerung oder -Verringerung in Prozenten bei Anwesenheit von 0.1-m Mds
Nierenbrei . . .	0.021	0.047	0.027	0.026	0.006	— 77	316	205	— 35
Leberschnitt . .	0.017	0.040	0.021	0.023	0.004	— 83	245	129	— 46
Nierenextrakt + Alanin . . .	0.070	0.225	0.100	0.155	0.030	— 81	448	543	+ 21
Leberextrakt + Alanin . . .	0.052	0.092	0.058	0.040	0.006	— 85	149	196	+ 32
Nierenextrakt + Prolin . . .	0.050	0.070	0.052	0.020	0.002	— 90	260	406	+ 56
Leberextrakt + Prolin . . .	0.040	0.057	0.045	0.017	0.005	— 70	321	[1]298	— 7

Aus der Tabelle geht hervor, dass Mds in einer Konzentration von 0.1 mol eine beträchtliche Hemmung auf die Ammoniakbildung ausübt, und zwar nicht bloss auf die spontane, sondern auch auf die durch Alaninzusatz gesteigerte. Die Hemmung liegt bei ungefähr 80 %. Auch in den Prolinversuchen zeigt sich eine schwache Ammoniakbildung, die sicherlich von Ammoniakbildung im Extrakt und nicht vom zugesetzten Prolin herrühren dürfte. Sie stimmt nämlich gut überein mit der Ammoniakmenge, die in Spontanversuchen ohne jeden Aminosäurezusatz entstand.

Ein Vergleich der Ammoniakbildung im Nieren- und Leber*extrakt* mit *Alanin*-zusatz zeigt eine bedeutend stärkere Desaminierung bei Niere als bei Leber, obwohl das Nierenextrakt, wie gewöhnlich, aus ungefähr der halben Menge Organgewebe bereitet wurde, verglichen mit dem Leberextrakt.

[1] Mit 0.01-m Mds war der Verbrauch 368 cmm. Im Verhältnis hierzu ergibt sich eine Mehrung des Sauerstoffverbrauchs von + 15 %.

Ein Vergleich zwischen der Hemmung einerseits in der *Ammoniakbildung*, anderseits im *Sauerstoffverbrauch*, wie sie in den Versuchen mit *Nierenbrei* und Leber*schnitt* beobachtet wurden, zeigt, dass die Ammoniakbildung, prozentual gesehen, bedeutend stärker gehemmt ist als die Oxydation. Dies kann dafür sprechen, dass wirklich eine mässige Oxydation der Mds stattfindet und die durch Mds gehemmte Verbrennung etwas kompensiert. Die Verhältnisse können auch dafür sprechen, dass die Oxydationsprozesse, die von der Mds gehemmt werden, einen geringeren Teil des gesamten Sauerstoffverbrauchs des Gewebes ausmachen, dass aber diejenigen, die gehemmt werden, nämlich die Oxydation der Aminosäuren, stark gehemmt werden.

In den *Extrakt*versuchen liegt in der Regel eine Mehrung der Oxydation bei Anwesenheit von Mds vor, was bereits besprochen wurde. Nur im Versuch mit *Leberextrakt und Prolin* findet sich eine leichte Minderung, die jedoch im gleichen Versuch mit schwächerer Mds nicht auftrat. Sie darf daher als eine in die Länge gezogene initiale Hemmung der stärkeren Mds-Konzentration gedeutet werden; auch dies wurde schon oben behandelt.

Um die Hemmung zu untersuchen, die Mds in schwächerer Konzentration auf die Ammoniakbildung ausüben kann, wurden diesbezüglich Versuche mit Nieren*extrakt* und *Alanin* angestellt. Wie aus Tabelle 3 hervorgeht, macht die Hemmung schon bei einer Konzentration von 0.01 mol ungefähr 20 % aus und steigt allmählich mit dem Steigen der Mds-Konzentration.

Tabelle 3.

Spontanversuch		Spontanversuch + 0.1-mol Mds		Spontanversuch + 0.05-mol Mds		Spontanversuch + 0.025-mol Mds		Spontanversuch + 0.01-mol Mds	
mg H_3N	% Hemmung	mg M_3N	% Hemmung	mg H_3N	% Hemmung	mg H_3N	% Hemmung	mg H_3N	% Hemmung
0.172	0	0.028	84	0.070	59	0.105	39	0.138	20

In diesem Versuch war die Ammoniakmenge in der *Blindprobe* 0.060 mg; dieser Wert wurde abgezogen, um die in der Tabelle angegebenen Werte zu erhalten.

Als *Schlussergebnis* dieser Versuche kann also angegeben werden, dass Mandelsäure die Oxydation sowohl im Nieren- als auch im Lebergewebe hemmte, und dass diese Hemmung mindestens teil-

weise durch eine Hemmung der Desaminierung gewisser Aminosäuren verursacht wird. Es ist indessen denkbar, dass Mandelsäure auch auf andere Prozesse vergiftend wirkt, wofür z. B. ihre Hemmung der Prolinoxydation in Versuchen mit Nieren- und Leberschnitt spricht. Aus den Versuchen geht nicht hervor, ob die Mandelsäure oder etwa eines oder einige ihrer Abbauprodukte dabei wirksam sind. Diese Fragen sind zur Behandlung in weiteren Versuchen aufgenommen.

Die in der Einleitung gebrachte Annahme, bei Mandelsäuretherapie sei die Ammoniakabwehr nicht genügend wirksam, wird also durch die angestellten Versuche gestützt. Auch diese Frage betreffend müssen aber noch ergänzende Versuche, vor allem an Gewebe von menschlichen Organen, gemacht werden, wenn eine sicherere Antwort möglich werden soll.

Zusammenfassung.

Einleitend wird besprochen, dass Anlass zu dem Verdacht besteht, Mandelsäure hemme die Desaminierung gewisser Aminosäuren und damit auch deren Oxydation. Dies könnte eine Erklärung dafür bilden, dass in gewissen Fällen bei intensiver Mandelsäuretherapie eine nur geringe Ammoniakbildung beobachtet wird.

In vor allem Nieren- und Lebergewebe von weissen Ratten wird mit Warburg-Technik die Oxydation mit und ohne Mandelsäure in verschiedener Konzentration sowie mit und ohne die Aminosäuren Alanin und Prolin untersucht. Weiterhin wird die Desaminierung mit Hilfe von Bestimmung des während der Versuche gebildeten Ammoniaks studiert.

Die Untersuchungen haben gezeigt 1.) dass Mandelsäure sowohl von Nieren- als auch von Lebergewebe verbrannt wird, jedoch stärker vom Nierengewebe; 2.) dass Mandelsäure die spontane Oxydation sowohl im Nieren- als auch im Lebergewebe und ebenso die Oxydation von zugesetztem Alanin und Prolin hemmt; sowie 3.) dass Mandelsäure als ein starkes Desaminierungsgift wirkt.

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The Localization of the Adenylic Acids in Striated Muscle-Fibres.

By

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Of the groups of compounds that go to build up the main part of the musculature, only two have a marked selective light absorption in ultra-violet between 2 000 and 3 000 Å, namely the purine derivatives and the proteins. Of the first-mentioned, the adenylic acid and the adenyl pyrophosphoric acid constitute more than 95 %. The pyrimidine nucleus in these latter causes a pronounced absorption-band at 2 600 Å. The selective absorption of the albuminous substances is conditioned by tyrosin and tryptophan. These amino-acids both have absorption-bands around 2 800 Å. The average ultra-violet absorption of the muscle-tissue will consequently be predominantly determined by the sum of the absorptions of the adenine derivatives, the tyrosin and the tryptophan. Owing to the high specific absorption of the adenylic acid — at 2 600 Å it is more than 100 times higher than that of myosin — its absorption-band will have approximately the same height as that of the muscle-protein in spite of the low concentration. (Cf. below, fig. 1.).

Investigations carried out by different authors on material from widely differing animal-species show an extensive agreement in the adenine content as well as in the tyrosin and tryptophan content of the muscle-protein (BAILEY 1937), so that there seems to be justification for the assumption that the main features of the structure are the same for large groups of animals.

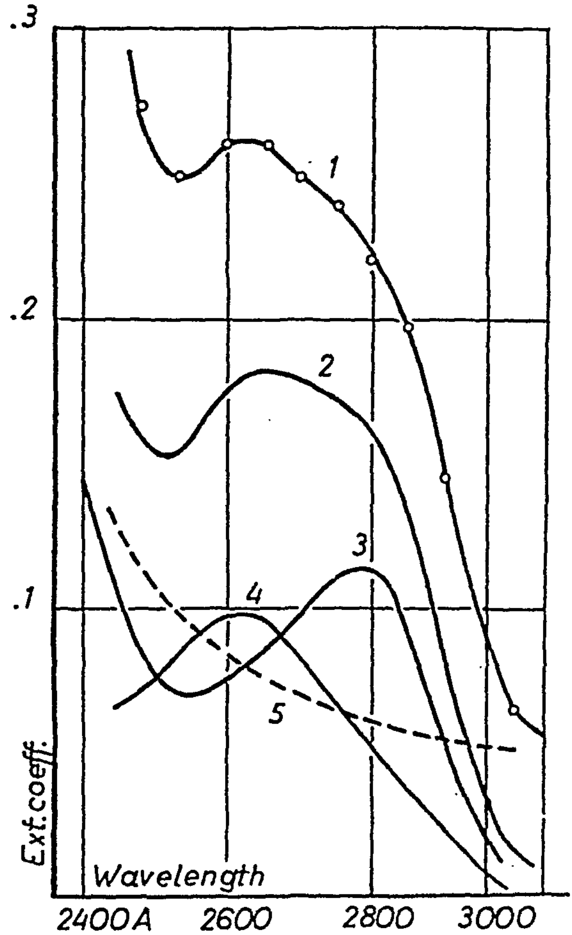


Fig. 1. Curve 1, absorption-spectrum of living muscle-fibre from Drosophila funebris, taken with so little resolving power that the striation has no influence on the course of the curve (cf. text, page 97). 2, the same absorption-spectrum after correction has been made for light-dispersion (curve 5 has been subtracted). 3 and 4, the absorption-curves for myosin and adenylic acid respectively, in which curve 2 can be resolved. 5, the course of the light-dispersion, calculated on the assumption that at 3 100 Å this conditions the whole absorption and is inversely proportional to the fourth power of the wavelength.

Illustration 1 shows an absorption-spectrum of muscle-fibres from Drosophila funebris, taken with the technique described below (cf. page 99). The absorption-curve shows a pronounced maximum at 2 600 Å. Above this is superposed a maximum at 2 800 Å, appearing chiefly as an increase in breadth of the first-mentioned max. The course of the absorption-spectrum of the adenylic acids is known (DHÉRÉ 1906, GULLAND and HOLIDAY 1934, HEYROTH and LOOFBUROUW 1931) and the myosin can be calculated (cf. CASPERSSON 1940). The measured curve can then easily be resolved into the two component curves (cf. CASPERSSON loc. cit.), as has been carried out on fig. 1. In order to make such a resolution into two components one needs only two points. The fact that the agreement in all curve-points is good shows that no substances with other absorption-character than those of the adenine and the protein markedly affect the absorption of the muscle-tissue. The concentration-relation adenylic acid to protein of the absorption-type of the myosin, which may

be calculated from the curves, is very near 1 : 100 in good agreement with the chemical investigations of musculature from different animals which give as an average 10—20 % protein and 0.1—0.2 % adenylic acid.

At the adenine absorption-maximum the ultra-violet microphotograph of the living muscle-fibre (Drosophila) clearly shows the striated structure, in that certain segments absorb more than others (illustration 2). It is not possible to decide off-hand whether we have here to do with a refraction phenomenon or a true absorption. If photographs of the same living muscle-fibre are taken with a *wide aperture for the illuminating bunch of rays* one observes the following: the striated structure appears between 2 400 Å and 2 950 Å — most clearly between 2 500 and 2 850 Å. Under 2 400 Å the absorption in both segments is so high that the structure appears only as an indication; over 2 950 Å the absorption in both segments is so low that the picture of the structure begins to disappear. Such series have been performed on musculature from frog, fresh-water crayfish and the fruit-fly, with identical results. They thus show preliminarily that the striation in the ultra-violet picture must be at least partly conditioned by real light-absorption, and not only by refraction-phenomena as in visible parts of the spectrum. It appears, moreover, that the absorptions in the different muscle-segments change in different ways with the wave-length. In order, however, to decide which substances condition the absorption, *complete absorption-spectra must be measured for the different structural elements.*

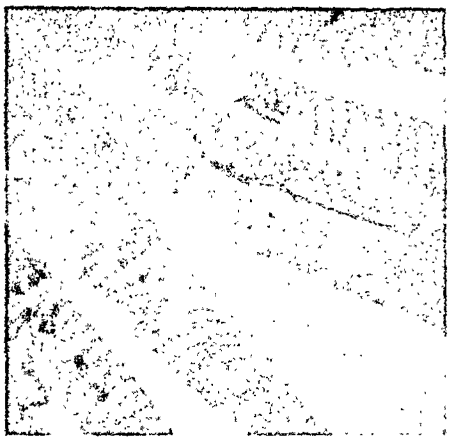


Fig. 2. Living muscle-fibres from the thigh-musculature of Drosophila funebris photographed at 2 570 Å. Enlarged 720 times.

A method for the measurement of absorption-spectra of small objects has been worked out by one of the present authors and has been described elsewhere (CASPERSSON 1936, 1940). The lower limit for the magnitude of the element that can be measured is determined by the wave-length of the light employed. In order to obtain a detailed absorption-spectrum the magnitude should not

be less than four times the wave-length of the light, as the light-diffraction will otherwise introduce a factor that is difficult to calculate. The measuring is considerably facilitated and accuracy increased if the dimensions are somewhat greater. As striated musculature in general has such short segments that the above-mentioned value is approached, it is most suitable to look for material with especially long segments.

Investigations on Drosophila funebris.

For these investigations muscle-fibres from coxa, trochanter and femur were used. This musculature has segments of up to 10 to 15 μ. The appearance at 2 570 Å of the living muscle-fibre may be seen in fig. 2. Certain segments absorb strongly. The nuclei, which lie within an axial zone, contain isolated grains of strongly absorbing substance (nucleic acids).

As the measuring takes a comparatively long time, during which the preparation must be quite motionless and unchanged, it is not possible to measure living material; the preparation must first be treated with an appropriate fixing fluid. This must fulfil the following requirements: 1) The distribution of the absorbing substances that are to be investigated must not be noticeably changed. 2) The protein substances must not be precipitated so that surfaces entailing an abrupt alteration in the refractive index can arise. Owing to the impossibility of defining the physico-chemical conditions in the muscle-fibre of the substances in question, model experiments are of but slight value. Instead, a direct comparison was made in ultraviolet of the living muscle-fibre with that which had been treated with fixative. A series of different combinations were tried, the best results being obtained with 25 % acetic acid in absolute alcohol saturated with lanthane acetate. In this fixative a muscle-fibre is not noticeably changed in the aspects referred to in the course of twenty-four hours.

When measuring, a region with an approximate diameter of 1 μ was projected into the photo-electric cell. Such measuring-points have been marked in fig. 3. By means of a special arrangement it is possible to move the preparation and afterwards return it to its original position with a smaller error than corresponds to the magnitude of the smallest element that the objective can

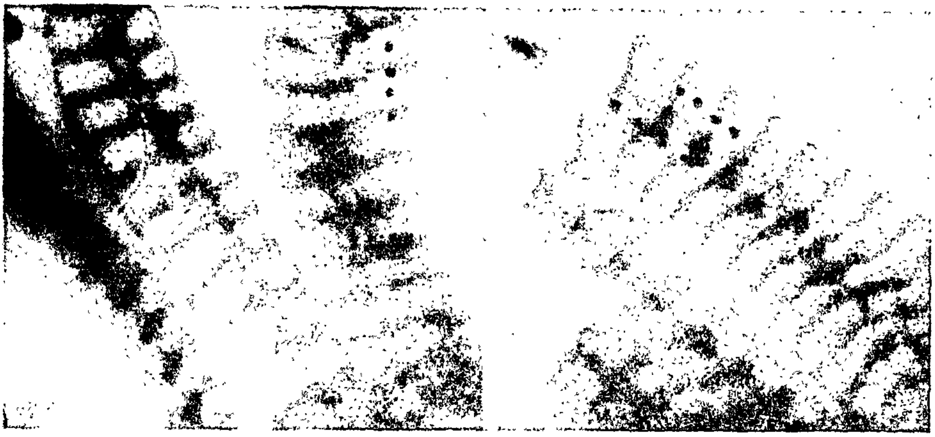


Fig. 3. Muscle-fibres from Drosophila. The dots mark the position and approximate size of the fields that in connection with the measurement were projected into the photo-cell. 2 570 Å 720 times. A pair of curves from each fibre are to be found in *figure 4*.

resolve, i. e. 0.05 μ. The selected measuring regions are projected into the photo-cell in turn. Measuring takes place in wave-length after wave-length, as a rule from 2 480 Å to 3 100 Å.

Little-Working Musculature.

The preparation was made from young flies from flask cultures. The flies were anaesthetized with ether and dissected, after which the preparation was immediately transferred to the fixative or was examined at once in the body-fluid.

Figure 4 shows absorption-curves from three different muscle-fibres. In each part-figure are drawn 2 curves (I and A respectively), deriving from two adjacent segments. All show a strong adenine band in the one segment, while this is only weakly represented or entirely missing in the other segment. In the more weakly absorbing segment the protein band appears clearly. There is a protein-band of the same magnitude also in the other segment; but this appears only as an increase in breadth or bulging of the adenine maximum. In some muscle-fibres also the more weakly absorbing segment shows an adenine absorption of varying strength (cf. fig. 4 c). This is, however, always considerably lower than in the adjacent segment.

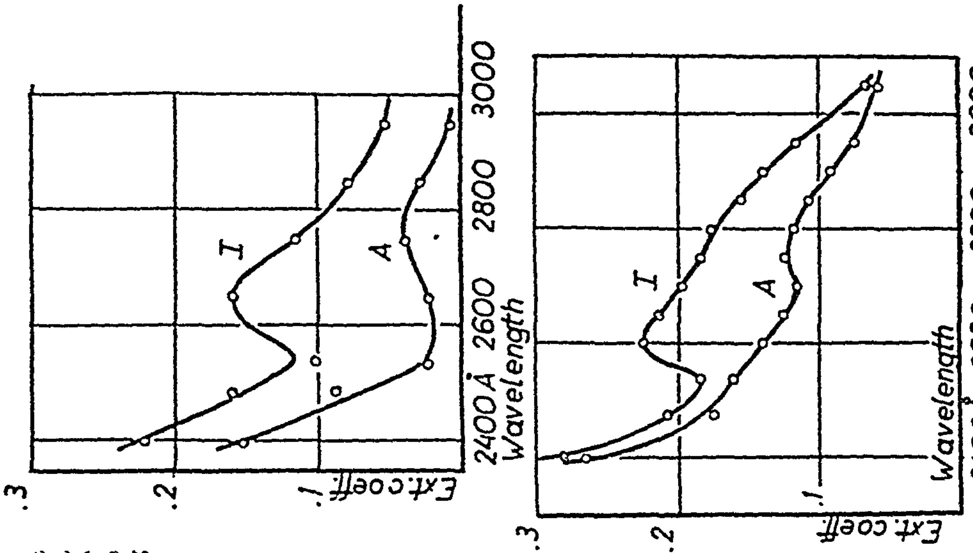
Table of quotients between the extinction-coefficients for the wavelengths 2 650 Å and 2 850 Å for points in different segments.

Isotropic segment	Adjacent anisotropic segment
1.48	1.03
1.44	1.27
1.47	1.22
1.49	0.96
1.59	1.17
1.45	1.37
1.42	1.14
2.00	1.42
1.83	1.24
1.39	1.17
1.33	0.97
1.92	1.15
1.61	1.11
1.66	1.18
1.85	1.00
1.80	1.28

In order to demonstrate the magnitude of the variations in the ratio archive the table gives a series of quotients between the extinction coefficients at 2 650 and 2 850 Å for pairs of adjacent segments from different muscle-fibres in different preparations. The value of this quotient for pure adenylic acid is 2.3 and for a protein of the composition of myosin 0.9 (cf. fig. 1). These quotients have throughout a higher value for the segments that in the table are referred to under I, which shows that these are richer in adenine nucleotides.

From these quotients we can calculate the approximate relation between adenylic acids and protein of the myosin type. These relations are illustrated in figure 5. (Cf., however, the paragraph below.)

In calculating the absolute quantities of adenylic acid and proteins from the measuring data one must also observe the loss of light arising through light-dispersion. In the complete absorption-spectra it is possible to introduce quite a good correction for this, as this loss of light is approximately inversely proportional to the fourth power of the wavelength. If there were no such dispersion of light the ultra-violet absorption would correspond to that of solutions of the substances in question and thus over 3 000 Å fall to a low value. In the muscle-fibres, however, a weak absorption still appears even at 3 200 Å, which must be due to



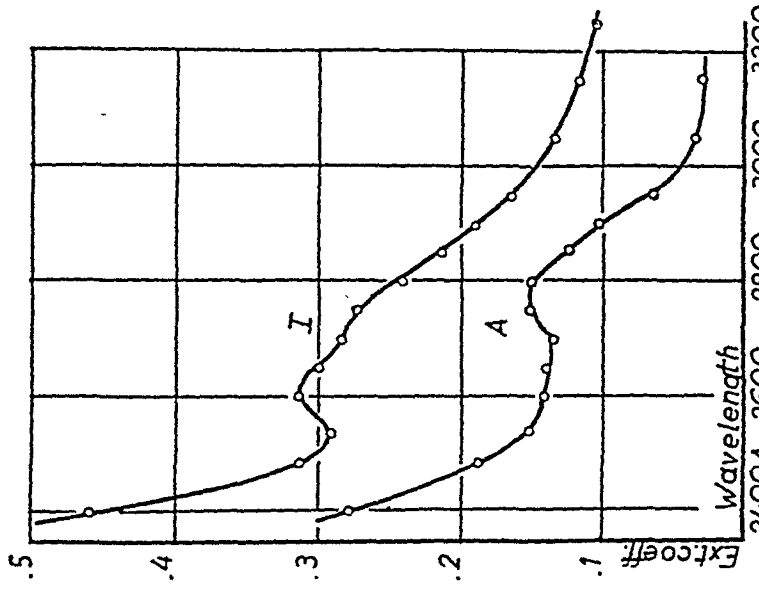


Fig. 4. Absorption spectra from strongly ultra-violet absorbing (isotropic) segments and weakly ultra-violet absorbing (anisotropic) segments lying adjacent to one another (cf. fig. 3). Three different muscle-fibres. The segments are marked I and A respectively according to the double-refractive conditions.

light-dispersion. As the wave-length relation of the latter is known one can introduce a correction for this (see curve 5, fig. 1). This correction cannot, of course, be introduced for the quotients given above, so that the value of the latter is limited to affording a support to the general validity of the data given above for a large material and a demonstration of the range of their variation. The error that is introduced in the quotients through dispersion of light entails in the quotients a stronger rise of the extinction coefficients at the shorter wave-length and affects most the

A-segment. It will thus considerably affect the result in *a direction contrary to the expected effect*, so that it cannot falsify the result.

The spiral arrangement in the muscle-fibres also complicates the measuring. This arrangement can be observed in at least large parts of the muscle-fibres, and it often makes it difficult to get muscle-partitions freely projected from adjacent segments. The error that may be conceived to arise goes in the same direction as the previous one, i. e. it tends erroneously to equalize the concentration differences.

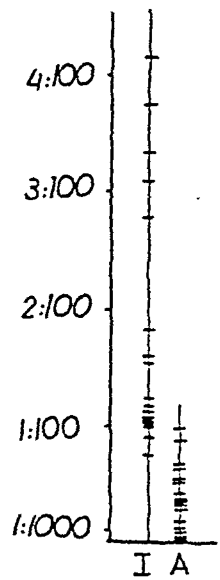


Fig. 5. The relation of adenylic acid to protein in different segments, corresponding to the quotients in the table, calculated without correction for the light-dispersion. Although the light-dispersion has a strong tendency apparently to equalize the differences (cf. text), the big differences may be clearly seen.

Absorption-spectra and the quotients given in the tables thus indicate that the greater part of the adenine derivatives are localized to the segments with the strongest ultra-violet absorption. In the majority of the muscle-fibres with especially long segments that were measured, the concentration-relation was considerably below 1 to 10 (calculated after correction for light-dispersion). The protein concentrations cannot be compared with any high degree of accuracy. Certain, there are reasons for assuming that the distribution of amino-acids in myosin is fairly homogeneous in the animal kingdom, but we have not sufficient experimental data for the other protein substances. If we assume that these have approximately the same tyrosin and tryptophan content as myosin (as seems to be the case for rabbit musculature), the protein concentration in the weaker absorbing segments of Drosophila muscle would be somewhat higher than in the other segments. The greatest difference that was observed corresponds to approximately 3 : 2. When the adenine absorption has been subtracted also the absorption under 2 500 Å gives a measure of the protein concentration that however is not alone determined by the tyrosin and tryptophan content. Owing to the non-selective character of this part of the curve, this calculation, too, will include considerable errors. It gives however, approximately the same values as the estimation of protein concentration given above.

Comparison between Ultra-Violet Absorption and Double Refraction.

In order to decide what structural elements observable with other methods correspond to the segments rich in adenylic acid, living muscle-fibres were photographed in both ultra-violet and polarized light. Comparison of the plates showed that *the strongly ultra-violet absorbing substances were localized in the isotropic segments.*

Fig. 6. Living muscle-fibre from Astacus between crossed Nicol's prisms. With the aid of the fine details in the original, which appear too weakly on reproduction, comparisons may be made with the corresponding ultra-violet picture, when it will be seen that the strongly absorbing segments correspond to the isotropic segments. 720 times.

Working Musculature.

The muscle-material has in all the cases so far discussed derived from flies in narrow culture-flasks. Cultivation took place at room-temperature. Under these conditions the flies alternately hop and fly short distances. The best material for experimental purposes was obtained from young individuals whose skin had just commenced to harden. These do not move about much. Before dissection they were anaesthetized with ether or chloroform. The muscles may thus be regarded as working relatively little. In order to make a comparison with especially strongly working musculature the flies were made to perform as much work as possible with their leg muscles. Faradic stimulus was applied so that the flies, deprived of their wings, had to move about on a plate with two comb-shaped electrodes, whose teeth interlocked and which were connected up with a source of current. The stimulus was so strong that the flies sprang several mm. into the air when they contacted it. A stimulus of a more physiological kind was obtained by letting the flies move about on a plane disc whose slope towards the horizontal plane was continuously changed. In this way they could be kept in continuous movement for several minutes.

The muscle-preparations were made in the same way as described above, and were examined both in the living and in the fixed states. The structure showed a constant difference as com-

pared with the resting muscle-fibres in that the sharp differentiation of strongly and weakly absorbing bands was more or less smudged out (Fig. 7). The strongly absorbing segments at lower

Fig. 7. Two pairs of pictures taken from living muscle-fibres from Drosophila at 2 570 Å and showing in low and high enlargement respectively the difference between strongly working (left pictures) and feebly working (right pictures) muscle-fibres.

magnification appeared to be increased in heigth and diffusely delimited. The structure is never quite wiped out. At high magnitudes the absorbing material in the resting muscle gives the appearance of rows of small irregular heaps of grains lying close together. The size of the single grains is near the limit of the re-

solving power of the ultra-violet microscope. In the fatigued muscle the anisotropic segments are extremely finely longitudinally striated. The striation appears most clearly nearest to I and diminishes in clarity towards the middle of A. The structure is conditioned by extremely fine streaks of strongly ultra-violet absorbing substance, which are connected with the heaps of grains in I. Probably the only explanation of how such a picture could arise is that strongly absorbing substances from I have wandered down towards the middle of A through a system of very fine longitudinal fibrils. As the absorption-character of the substances agrees on the ultra-violet photographs with that of the substance of the I-segments, and despite their fineness the striae contrast sharply with the surrounding protein, the specific extinctions must lie much higher than for the protein-substance in A, and be comparable with those of the adenine derivatives in I. It must thus be the adenine derivatives in I that in the course of muscle-activity partly wander down towards A. This probably also explains the circumstance that certain of the abovementioned quotients and absorption-spectra from only slightly working musculature show a varying though low content of these substances in A.

In experiments of this type it is difficult to avoid artefacts. In order to exclude such we investigated unfixed muscle-fibres that had been left to lie in body-fluid or in Ringer's solution for varying periods. When muscle-fibres had been thus left for a longer period, pictures of an extension of absorbing substances from I to A also appeared. The streaks that appear in this connection are, however, coarse and crude, and at the same time one observes changes in the fine structure of I. In connection with attempts to find suitable fixing media (page 100) these last-mentioned pictures were also observed. There is thus a very definite difference between the pictures of fine striae that appear in the fatigued muscle and are lacking in the resting muscle, and the pictures that are observed in both resting and fatigued muscle-fibres when the preparation is left to lie for a long time or is treated with unphysiological solutions. It thus seems justified to assume that what has been described above cannot be referred to artefacts, but that in working musculature the adenine derivatives wanders down towards A.

Investigations of Other Musculature.

In order to decide in how far the observations made with reference to Drosophila funebris possess general validity certain other musculature was also examined.

Astacus fluviatilis.

In parts of its musculature the fresh-water crayfish has muscle-segments of magnitudes comparable with those found in Drosophila Muscle-fibres from the dorsal hypodermal muscle-layer were examined. Ultra-violet micro-photographs showed the same typical arrangement as has been described above for Drosophila. A series of quotients were taken, also in the same way as above-described and with the same result as for Drosophila. The polarization investigation showed that the absorbing substance lay collected in the isotropic segments. (Fig. 6.)

Ascaris megalocephala.

In the extremely large sub-epidermal muscle-cells of Ascaris only a smaller part of the cytoplasm is differentiated as myofibrillae (cf. fig. 8). Here then the possibility presents itself of examining in one and the same cell equally thick layers of differentiated and non-differentiated cytoplasm and to measure absorption-spectra with moderate enlargements. The fibrilla-part has a strong ultra-violet absorption. Absorption-spectra therein show a high concentration of substances with purine absorption as well as a high protein concentration, fully comparable with the musculature of the insects and vertebrates. In the fibrilla-free part, on the other hand, there are only extremely small quantities of substances with the 2 600-band, so that the protein-band appears almost pure (fig. 9). The protein concentration is also rather lower in these parts.

Fig. 8. Parts of two muscle-cells from the sub-epidermal musclelayers of Ascaris. The peripheral layers differentiated. Wavelength 2 570 Å. Enlarged 300 times.

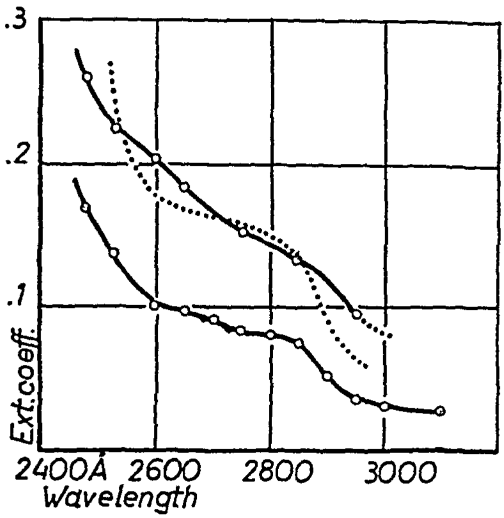


Fig. 9. Absorption-spectra of points in the differentiated (upper continous curve) and undifferentiated parts (lower continous curve) of muscle-cells from Ascaris. The dotted curve gives the values of the lower curve, multiplied by an arbitrary factor. Comparison with the curve from the differentiated part shows, even without correction for light-dispersion, that the absorption of this part is proportionally higher than that of the undifferentiated part around 2 600 Å (the adenylic acid band) but lower around 2 800 Å (the protein band) and below 2 500 Å (where the protein absorption rapidly rises while the adenylic acid absorption sinks). From this it appears that the differentiated part contains much more adenylic acid than the other part.

Vertebrates.

Striated musculature from frog, rat and dog was examined with ultra-violet micro-photography. The arrangement of alternately strongly and weakly absorbing segments appears clearly. Fig. 10 shows a living muscle-fibre from a frog. That here, also, despite the smallness of the structure, we have to do with a true absorption and not only with light-refraction appears from serial photographs in different wave-lengths, which show the same conditions as have been described above for Drosophila musculature. Owing to the shortness of the muscle-segments and the great thickness of the muscle-fibres in comparison therewith no attempts were made to work out detailed absorption-spectra.

In order to compare the relation between double-refracting and ultra-violet absorbing segments finely pulverized asbestos was sprinkled over the preparation of the living muscle. On the ultra-violet photographs and the plates that were taken in polarized light it was then possible to identify isolated segments with the guidance of the sharpedged, double-refracting grains of asbestos.

Here, too, the vaguely double-refracting and the strongly ultra-violet absorbing segments were in agreement. (In certain material also a very fine strongly absorbing line goes through the central part of the strongly double refracting segment.)

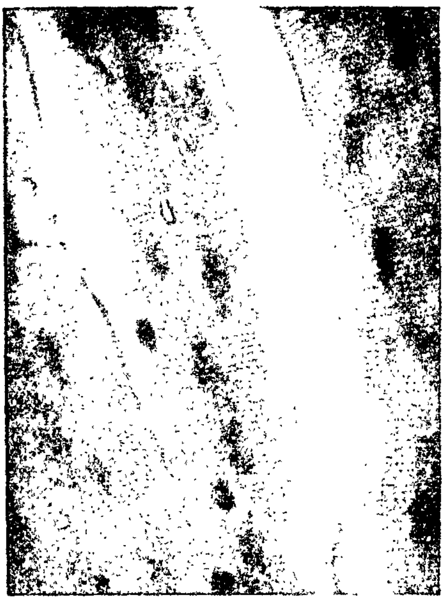


Fig. 10. Living muscle-fibres from frog, photographed in body fluid at 2 570 Å. Enlarged 720 times.

Cardiac musculature also shows the typical arrangement of strongly and weakly absorbing bands. In the fibres of Purkinje, however, there is striation only in the outermost layers and there merely as an indication. Absorption-spectra were measured from the central region of Purkinje's fibres (calf) and from the adjacent musculature. The preparation was 5 μ in thickness and the diameter of the region that was projected into the aperture of the photo-cell about 5 μ. The muscle-segments were considerably lower than that value and were passed obliquely by the light-rays. The muscle-structure was thus not "resolved" by the photo-cell. As the light-absorption is dependent solely on the *amount* of substance that the ray of light passes through and is not affected by the distribution of the absorbing substance, the *absorption-curve in this case will be approximately the same as it would be if the substances were in molecular solution.* The only condition that must be fulfilled if the structure-factor is to be eliminated in this fashion is that the structure-elements shall be numerous and so small in comparison with the magnitude of the measured region that the different parts of the photo-cell receive more or less equal amounts of light. Figure 11 shows absorption-curves. They show that Purkinje's fibres lack the high concentrations of adenine derivative that are characteristic of the contractile muscle-cells.

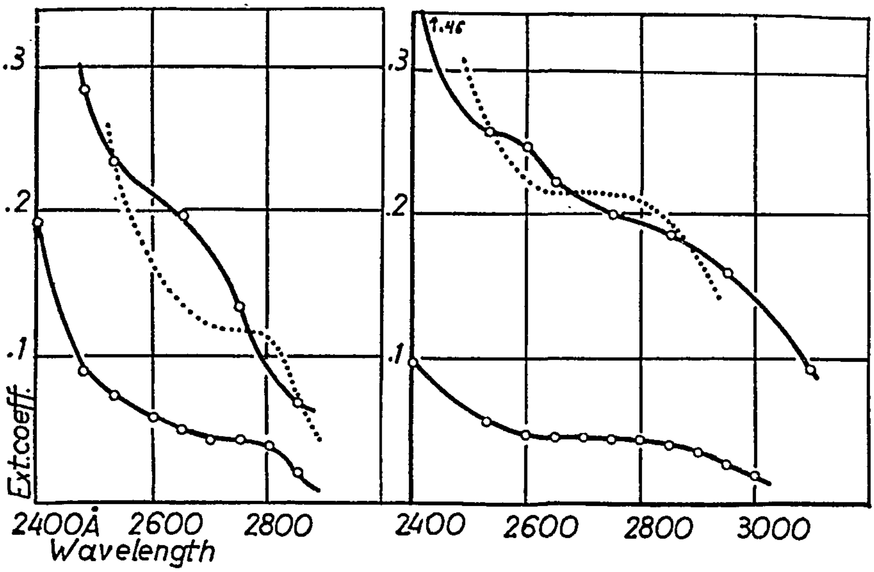


Fig. 11. Two pairs of curves from points in Purkinje's fibres (the lower continous curves) and from adjacent cardiac musculature (the upper continous curves). The dotted curves have been plotted in the same way as in figure 8 and show the same conditions as there.

Survey of the Results.

The ultra-violet absorption exercised by the adenine derivatives at 2 600 Å is as compared with protein substances of the muscle-protein type exceedingly high — the order of magnitude of the extinction-coefficient relation is about 100 : 1; in consequence, elements that are rich in adenine derivative will appear clearly in ultra-violet pictures taken at this wave-length even if large quantities of protein are in the immediate vicinity. Owing to the dispersion of light in the muscle-structure, that is composed of elements with considerably different refractive index, such pictures are, however, far from unambiguous.

As compared with other tissues the musculature is distinguished by great quantities of adenylic acids. In order to demonstrate that these are for the most part combined with the contractile elements, absorption-spectra of the epithelial muscle-cells from *Ascaris* were measured, where only a part of the cytoplasm is differentiated to form myofibrillae. The fibril-part showed a high concentration of substances with the adenine-band (as well as fairly high protein concentrations), while the fibril-free cytoplasm showed a pure protein spectrum. A similar comparison is offered

by PURKINJE's fibres in the mammalian heart. In the calf they possess only a thin layer superficially differentiated to myofibrillae. Absorption-spectra of the part poor in fibrillae show a very low content of substances with selective absorption about 2 600 Å, while the cells of the adjacent cardiac muscle-tissue with the same layer-thickness show a high band in this place.

By measuring complete ultra-violet absorption-spectra of the isotropic and the anisotropic parts in the musculature of arthropods with especially long segment it was possible to show that the main part of the adenine derivatives in non-working musculature lie collected in I. The partitions of the vertebrate musculature are too small to allow of an exact absorption-measurement; but the photographic series taken at various wave-lengths show with very great probability that the case is the same also here.

Attempts to compare fatigued and unfatigued musculature were carried out on Drosophila material, which has especially long segments. In the fatigued musculature substances containing adenylic acid were seen to wander down from the isotropic to the anisotropic segments.

Discussion.

A number of different circumstances speak strongly in favour of the assumption that myosin, first investigated in detail by EDSALL and MURALT, is the prime factor in muscle-contraction. It has been found in all examined muscle-substance, and its composition in widely varying classes of animals is strikingly homogeneous. (See BAILEY, 1937). That the long polypeptid chain of the myosin molecule, like that of keratin, can be stretched and contracted by folding of the molecule-chain has been demonstrated by ASTBURY and DICKINSON (1935). These workers were in one case even able to show such a folding in living musculature in connection with contraction.

Comparisons between the polarization-optical and Roentgen-optical qualities of myosin fibres and muscle (see WEBER) show that the double-refractive muscular part consists of myosin-rods arranged with complete axial parallelism. These rods have a diameter of about 45 Å and a length of at least 500 Å. The protein content in the single rod amounts to about 70 %. The rods are built up of at the most 20 fibre-molecules the involuted state of whose molecular chains can be changed. The double-refractive

muscle-segment thus constitutes an organ that is extremely highly specialized for the performance of a special task and in which for mechanical reasons there is but little room for other substances than the contraction-conditioning myosin. I is not in the strict sense isotropic; for SCHMIDT (1934) has shown that it has a double retraction that in the material investigated amounts to about 10 % of A's. It is probably conditioned by small quantities of myosin. Other protein substances must, however, be present, as is shown both by dessication experiments (NOLL and WEBER 1934) and the absorption-measurements given in the foregoing. The main part of the contraction takes place in A, while I's share has been discussed. STUDNITZ found that on a weak contraction I's height was not changed, though it diminished on strong contraction. Whether we have here to do with an active actual contraction or with a passive shortening brought about by A's change of form cannot be decided. The decrease in height is, even on strong contraction, less than that of the adjacent A. The rôle played by the isotropic segments in the mechanical process of contraction seems thus to be a subordinate one, or in any case less immediate than that of the anisotropic segments.

The glycolytic processes that constitute the energetic basis for the contraction seem in different types of muscle-tissue to follow a course similar to that found in the type of musculature that has been most carefully investigated, namely, skeletal muscle. (See regarding smooth musculature: DWORACZEK and BAARENSCHEEN, 1937; MEERAUS and LORBEER, 1937. Re cardiac musculature: OCHOA 1937). The amount of creatine phosphate varies. In certain muscles its task seems to be performed to a greater or lesser extent by arginin phosphate. Adenylic acid and adenyl-pyrophosphoric acid are invariably found.

Unambiguous data from various quarters show that the breaking up of the adenyl-pyrophosphoric acid, which reaction is exothermic and rapid, precedes the other processes in the breaking down of the carbohydrates. It probably takes place in the very first stage of the contraction (see e. g. LUNDSGAARD, 1938). The subsequent reactions: the breaking down of phospho-creatine and phospho-arginin etc. as well as the actual glycolysis, serve to restore the adenyl-pyrophosphoric acid and to maintain it at a constant phosphorylation level, which in the resting muscle seems to be high.

The demonstration in the foregoing that the adenine derivatives

in the resting muscle are chiefly localized in the isotropic segments shows that the segmentation of the striated musculature implies a chemical differentiation covering more than the differences in myosin-concentration that the polarization-optical investigations render probable. The most probable explanation is that the formation of the adenylpyrophosphoric acid (by far the most predominant adenine derivative in resting muscle) takes place in these. The striation of the muscle would thus be conditioned by a chemical differentiation in a contractile part and a part where the formation of substances that supply energy for the contraction takes place. There is also a certain support for such a view in earlier observations. That the staining conditions in I and A change in connection with the contraction has been known for long, but on account of the complicated physico-chemical conditions it seems at present impossible to draw any conclusions whatsoever regarding the course of the chemical processes. With glycogen-staining in resting muscle STUDNITZ (1934) found glycogen both in I and A. After work the stainable granules disappeared, and on restitution they reappeared first in I. This speaks in favour of a particularly important rôle for I in the glycogen conversion. The observations alone that during strenuous muscular work the adenine derivatives wander along the fine fibrillar structure into A must, since artefacts can in all probability be excluded, be interpreted as a direct transport of adenyl-pyrophosphoric acid, from I direct to the contractile elements, as this acid constitutes the main part, possibly all, of the adenine derivatives during rest. The energy from the glycolysis in I is thus transported direct by adenylic acids to the contractile protein chains and there used for the contraction. Whether this takes place as an introduction to a contraction or as a restitution after a contraction is irrelevant in the present discussion.

As regards the chemical process in connection with the transferance of energy from the adenyl-pyrophosphoric acid that has flowed down in Q and is in contact with the oriented myosin-rods, absorption-data cannot, of course, give any help. Certain chemical investigations seem, however, to show that such a direct transferance as has been postulated as most probable in the light of the above data, is chemically quite conceivable. ENGELHARDT and LJUBIMOWA (later confirmed by SZENT-GYÖRGYI and BANGA (1940) and D. M. NEEDHAM[1] found that *the ferment that splits*

[1] Not published, cited in J. NEEDHAM et collab. (1941).

the adenyl-pyrophosphoric acid cannot function except in the presence of myosin or else is myosin itself; and they maintain that "the mineralization of adeninetriphosphate, often regarded as the primary exothermic reaction in muscle contraction, proceeds under the influence and with direct participation of the protein considered to form the main basis of the contractile mechanism of the muscle fibre."

J. NEEDHAM, SHEN, D. M. NEEDHAM and LAWRENCE (1941) have further confirmed this in that they have shown that adenyl-pyrophosphate has a markedly strong capacity to decrease the double refraction of flow in a myosin solution. This effect, that is referred to a change in the degree of dispersity and the particle-length is, curiously enough, reversible. As a possible mechanism for the interaction between adenyl-pyrophosphate and myosin in the muscle these authors suggest the hypothesis that the myosin itself is phosphorylized and is the last link in the chain of simultaneous energy and phosphate transferance.

These observations, that show that a transferance of energy direct from adenyl-pyrophosphate to myosin is in a high degree probable, supplement well what has been shown above. If the myosin, or a therewith closely associated protein is responsible for the dephosphorylation and thus also the liberation of energy — and according to NEEDHAM, SHEN, NEEDHAM and LAWRENCE's hypothesis also responsible for the transferance of the energy by means of phosphorylation to the actual myosin molecules — then this process must take place above all where the main part of the myosin is localized, i. e. in the anisotropic segments. This constitutes the continuation of the process that has been postulated above on the strength of the cyto-chemical data, and it thus seems probable that the energy with adenyl-pyrophosphate is transported not only to but also on to the contractile myosin.

On account of the short transport-roads the diffusion after the existing concentration-falls is probably sufficient to explain the wandering of the released adenyl-pyrophosphate from I and of the adenylic acid in the opposite direction, which may also be a part of the explanation of the circumstance that musculature with short segments is capable of quicker action than is musculature with long segments.

Whether the muscle-contraction is released by the diffusing of the adenyl-pyrophosphates to the contractile myosin-chains (from which they are spatially separated) either by permeability changes

or some similar process, or whether this process is a restitution-process after contraction of a myosin-chain bearing potential energy can only be decided by kinetic investigations. The strict way in which the substances in the resting muscle are separated from one another makes the former process seem at present fairly probable.

The characteristic structure of the striated muscle thus seems to be conditioned by a differentiation in parts with a chiefly contractile function and parts whose chief functions are in the service of the chemical conversion. In connection with the contraction, energy is transported from the latter to the former by means of adenyl-pyrophosphate of the corresponding phosphatase, which may possibly be the myosin itself. This arrangement, with parts only a few μ in height, just enable an extraordinary effectivity and rapidity of function, which qualities are among the most prominent of the striated muscle-fibre.

Summary.

Ultra-violet absorption-spectra have been measured of individual isotropic and anisotropic segments in striated muscle-fibres from insects. By means of comparisons with ultra-violet microphotographs it is shown to be probable that the results obtained in this way are applicable to striated musculature in general. The absorption-curves show that the main part of the adenine derivatives (adenylic acid and adenyl-pyrophosphoric acid) in the resting muscle are localized in I. These segments seem to have their main function as the seat of chemical energetic processes while A is the seat of the contractile elements. From observations on fatigued muscle-fibres one deduces that energy is transferred from I to A by adenyl-pyrophosphate whose energy is released by the phosphatase that is bound to the myosin.

The background to the striation of the muscle-fibres thus seems to be a differentiation of contractile parts and chemically-working parts respectively. The transport of energy from the latter to the former is performed by adenyl-pyrophosphoric acid.

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The Photopic Spectrum of the Pigeon.

By

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(Received 18 April 1942.)

In a number of papers in this Journal (GRANIT, 1941—1942) I have described the spectral properties of receptors of different retinae, analyzed with the micro-electrode technique first used by GRANIT and SVAETICHIN (1939) with the frog's eye. In some types of retinae isolated elements have been obtained without difficulties, in others simultaneous activity of several units has been the rule. The eye of the pigeon belongs to the latter type, in fact, so much so that I never have succeeded in obtaining a restricted response from this retina with the same micro-electrodes which have given large single spikes from eyes of rats, guinea pigs, and cats, less regularly, well isolated spikes even from frogs which are not so favourable preparations as the mammals mentioned. It is tempting to believe that the ultimate cause behind this fact is that convergence towards the optic nerve, where recording takes place, is far less prominent in the pigeon's eye (CHIEWITZ, 1889) than in the others. This eye has both rods and cones but is dominated by cones (SCHULTZE, 1866).

The fact that a reasonable degree of isolation of the colour-sensitive elements could not be obtained with the pigeon prevented a detailed analysis of their spectral properties. Only the average photopic spectrum of the pigeon's eye has been obtained.

Methods.

The pigeons were anaesthetized with a solution of 0.1 cc Avertin in 1.0 cc urethane (emulsion). About 0.4 cc of this mixture was injected intraperitoneally. The head was fixed with pins, cornea and lens re-

moved, and the micro-electrode inserted under the preparation microscope. Spontaneous activity was mostly present. The discharge to illumination consisted of massed impulses, amplified and led to cathode ray and loudspeaker. The threshold was determined in the different wave-lengths by listening to the discharge and observing it on the screen. The inverse value at the threshold was calculated in per cent of the maximum.

Results.

The pigeons were light-adapted for 10—20 min. to the microscope lamp (2,400 m.c.) after which the return of sensitivity was followed for a number of wave-lengths. As in all other animals with both rods and cones the recovery of sensitivity followed in two steps, a first slowly rising phase during which the absorption of the cone system dominated, and a second phase during which a rapid and large rise of the sensitivity (1/energy) took place. The latter phase led to a particularly great increase in the region of 0.500 μ. This change has been illustrated in previous work (GRANIT, 1941 b, 1942 a). Suffice it here to take 2 curves which also illustrate variations in a single experiment. These are shown in fig. 1. The curve to the right was obtained immediately after light-adaptation but dark-adaptation was already making itself felt in the short wave-lengths before the experiment was completed. Somewhat later in the same experiment the whole curve has shifted to the left. Thus the pigeon has a Purkinje shift. Nevertheless it sometimes happens that the electrodes pick up the discharge from a region which does not dark-adapt in this manner but merely slowly and moderately increases in sensitivity, the photopic distribution in the spectrum all the time remaining constant. One has probably then struck a rod-free area. In the rod-free eye of the Greek tortoise (GRANIT, 1941 a) all places behave in this manner. But in the pigeon

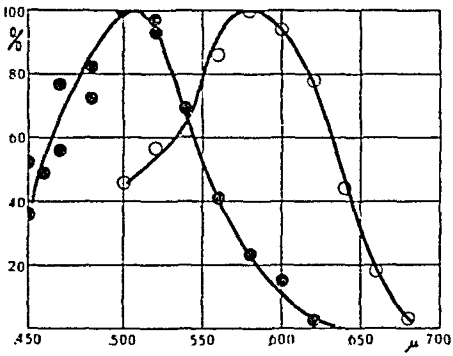

Fig. 1. Single experiment. Initially light-adapted pigeon first gave photopic distribution (open circles), later scotopic distribution of sensitivity (filled circles). Equal energy spectrum.

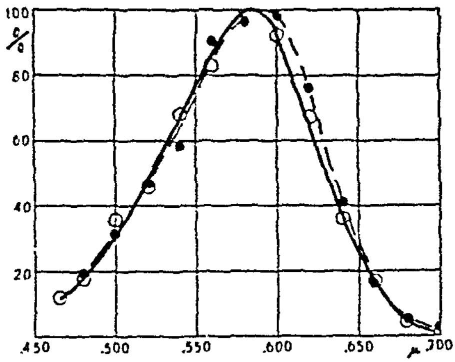

Fig. 2. Two experiments with light-adapted pigeons. Recording from places in the retina which did not dark-adapt in the course of the experiment. Equal energy spectrum.

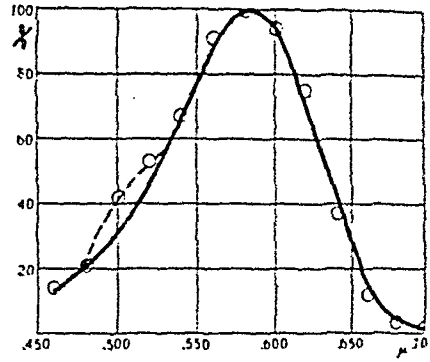

Fig. 3. Averages of 372 values from 27 series obtained from 20 light-adapted pigeons. Incipient dark-adaptation indicated by broken line. Spectrum of equal quantum intensity.

dark-adaptation as a rule begins after about 15—20 min. to affect the spectral distribution of the photopic eye. The beginning of this process is already found in the photopic curve of fig. 1.

These facts deserve to be pointed because methods based on training the animals, on pupillary reactions etc. cannot keep incipient dark-adaptation in check as well as the very much faster electro-physiological technique. The photopic maxima obtained by such methods can therefore easily become mixtures of photopic and scotopic curves. A photopic curve published by LAURENS (1923) with maximum in 0.564 μ is probably influenced by dark-adaptation.

In fig. 2 is illustrated two single experiments in which the increase in the short wave-lengths is absent. The maximum is found to be around 0.580 μ, or 0.020 μ further to the right than in the frog's eye.

We can then proceed to average the 27 series obtained from 20 pigeons, together based on 372 averaged values and a much greater number of individual readings. This curve, plotted against a spectrum of equal quantum intensity, is shown in fig. 3. The values are somewhat too high around 0.500 μ owing to the fact that it has not always been possible to avoid incipient dark-adaptation. Despite this the maximum must be placed around 0.580 μ. I should perhaps add that, excepting the easily recognizable tendency of the curves to be influenced by incipient dark-adaptation, I have never seen any animal in which the same photopic spectral distribution from case to case has recurred with such monotonous

regularity as the pigeon. Probably the reason for this is the great number of units that always were found to be simultaneously recorded. Every single curve is thus an average in itself.

Anomalous Nature of Purkinje Shift of the Pigeon.

One of the main conclusions suggested by my analysis of the colour receptors with microelectrodes (cf. esp. GRANIT, 1942 a) is that animals with good colour vision always have a broad dominator band of spectral sensitivity and a number of narrow and steep modulator bands in different regions. I have assumed (GRANIT, 1941 c) that the broad dominator band mediates the perception of white and that the modulators serve to modulate this impression to colour by emphasizing their own spectral regions when stimulated. The broad band, which in the frog (GRANIT, 1942 a) and in the cat (GRANIT, 1942 unpublished) also is served by isolated units is the "carrier" of the Purkinje shift. For the system activated by visual purple in scotopic vision (maximum around 0.500 μ) the photopic carrier of the Purkinje shift in frogs, cats and, to all appearance, also in man has its maximum around 0.560 μ. How are we now to understand that in the pigeon the dominator band, which in all animals is identical with the average photopic spectrum, turns up shifted 0.020 μ to the right with maximum around 0.580 μ?

There is, to begin with, no reason whatever to assume the visual purple of the pigeon to be different from that of the frog. Beyond the single set of readings in fig. 1 I have not made any systematic observations on dark-adapted eyes, for the simple reason that GRAHAM, KEMP and RIGGS (1935) already have provided such data with the electroretinogram as index. LAURENS (1923), utilizing the pupillary reaction, and GRAHAM, KEMP and RIGGS have come to the conclusion, supported also by my observations, that the scotopic photosensitive system reproduces the absorption curve for visual purple. Visual purple has been extracted from the eye of the pigeon already by HESS (1912).

Why then this apparent exception from what seems to be a rule, namely, that when the visual purple is of the type with maximum around 0.500 μ, then the photopic dominator substance has its maximum around 0.560 μ? Rather than to announce an exception from this rule, before compelled to do so by overwhelming evidence,

I would suggest that the difference is due to selective absorption by the intensely coloured oil globules of this eye. My assumption can be well supported by evidence.

The oil globules, discovered by HANNOVER (1840, 1843), were found by KÜHNE (1879) to consist of the red rhodophane, the yellowgreen chlorophane, and the orange xantophane. Their spectra have been determined by KRAUSE (1934), WALD and ZUSSMAN (1937) and by v. STUDNITZ and BUSCH (1941) in extracts from chicken retinae which possess the same droplets as the pigeon's retina. With the chicken there have also been carried out important experiments by HONIGMANN (1921) on the scotopic and photopic sensitivity distribution, one of the few papers on such subjects in which adequate training methods have been combined with adequate physical technique. He found in the dark-adapted state the distribution required by visual purple. In the light-adapted state the young animals were found to give a photopic curve with *maximum in* 0.560 μ, the fully grown animals, however, gave one with maximum around 0.580 μ. His curves are shown redrawn in percent of the maximum to the right in fig. 4.

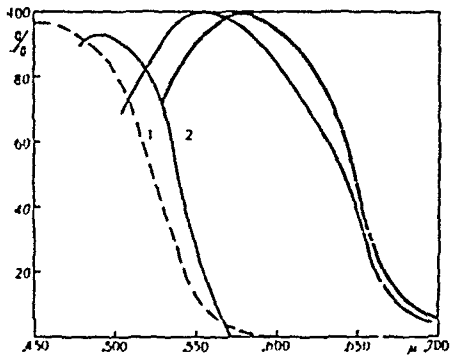

Fig. 4. *To the right:* HONIGMANN'S (1921) photopic curves for light-adapted chicken replotted on a percentage basis. The curve with maximum in 0.560 μ refers to young animals, the one with maximum in 0.580 μ to grown-up animals.
To the left: Absorption by the oil globules according to v. STUDNITZ and BUSCH (1941). 1, extract on all coloured droplets; 2, xantophane alone.

Now it is hardly probable that the photochemical substance has altered with age. His suggestion, that the density of the coloured filters has increased is altogether more reasonable. Their absorption maxima depend somewhat upon the solvent (see *e. g.* WALD and ZUSSMAN, 1937). I have inserted in fig. 4 two schematic curves, based on the data of v. STUDNITZ and BUSCH (1941), one for xantophane and the other for all substances extracted together with ether. It is clear that these filters tend to cut down sensitivity in the medium and short wave-lengths thereby pushing the photopic curve towards the red side. This gives a satisfactory explanation of the difference in the photopic curve of the pigeon as compared with the one found in frogs, cats and man.

WALD (1937) claims to have isolated from the eye of the chicken a substance which he calls »iodopsin» and assumes to be the cone substance with maximum in 0.580 μ. Quite apart from the fact that its spectrum cannot be regarded as settled by the work referred to, one would also like to reserve judgment as to the existence of this substance until further evidence has been presented.

Colour Vision of the Pigeon.

From the work of HAMILTON and COLEMAN (1933) we know that the pigeon has good colour vision, a conclusion which also is suggested by the presence of a Purkinje shift. It is regrettable that technical difficulties have prevented a study of restricted units of activity in this animal, but to judge from experiments on other animals with colour vision (GRANIT, 1941—1942) the broad dominator band of this eye must also be modulated by narrow bands in different regions. Perhaps the oil globules add somewhat to the differentation obtainable by these means.

Summary.

The precise form of the photopic spectrum of the pigeon has been measured with the electrophysiological technique used with other animals in previous contributions to this problem.

The filtering effect of the coloured oil globules has been discussed in relation to the photopic spectrum.

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Selective Activation of a Transient Reflex by Restricting Stimulation to Certain Frequencies.

By

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(Received 20 April 1942.)

There are some old observations by SHERRINGTON and SOWTON (1911) indicating that variations of form and strength of electric stimuli may lead to a certain amount of selectivity in the activation of the ensuing reflex pattern. But this line of approach to reflex excitation appears later to have been neglected to such an extent that, when we now took it up, it soon was possible to find a rather striking, regular and easily reproducible effect of a pure frequency variation into the higher ranges which with the aid of neon or thyratron stimulators now are available without expense and labour.

This effect consisted in a transient strong extensor reflex, coupled to a number of vegetative reflex reactions, maximal at a stimulation frequency around 100/sec. This paper will be devoted to a description and primary analysis of this phenomenon.

Method.

The experiments have been carried out on 25 decerebrated and 2 anaesthetized cats. The latter received 0.4—0.5 cc 10 per cent Dial Ciba per kg intraperitoneally. For stimulation neon-stimulators ranging up to 1000/sec. at constant output strength, were available. Extensor and flexor reflexes (mm. quadriceps and tib. ant.) were recorded with a BROWN-SCHUSTER spring myograph and kymograph. Only decerebrated animals were used in these experiments. Blood pressure and pupil

reactions were studied on the animals which received dial. The width of the pupil was recorded with a film camera (10 pictures/sec.) with the eye illuminated at a distance of 20 cm.

In the decerebrate animals all nerves except those innervating the muscles used were severed in most experiments.

Results.

The Transient Extensor Reflex against a Background of Crossed Extension.

The experimental arrangement was the one generally used for the recording of an ipsilateral inhibition (in this case through the proximal stump of the left sciatic) of a crossed extensor

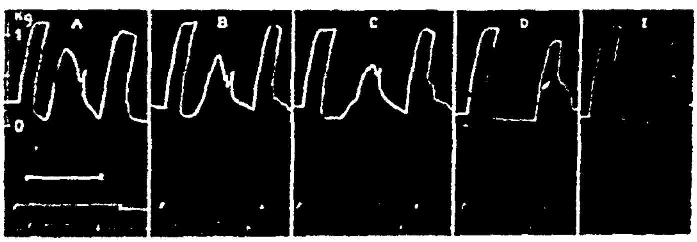

Fig. 1. Reflex contraction of quadriceps muscle. In this and the following figures records not strictly, but almost isometric. Duration of stimulation marked below. Upper line: stimulation of contralateral sciatic at 25 per sec. Lower line: stimulation of ipsilateral sciatic, causing inhibition succeeded by transient reflex, at frequencies 96 (A), 83 (B), 60 (C), 30 (D) and 15 (E) per sec. Inhibition alone and not followed by transient reflex in D and E. Horisontal line in A marks 20 sec.

reflex elicited by stimulation of the proximal stump of the right sciatic. The stimulus for the crossed extensor is of constant strength and frequency, the ipsilateral inhibitory stimulus is varied with respect to frequency. The frequency of the latter falls from A to E in fig. 1.

At the low frequencies (15 and 30 per sec.) D and E there is the typical well-known inhibition of the crossed extensor. But already in C at a rate of 60/sec. the inhibition is interrupted by a transient rise of excitation. In B, at 80/sec. this effect is better marked but still transient although, as the figure shows, the inhibitory stimulus still is active for some time after cessation of the transitory extensor contraction. This contraction is maximal in A with a stimulus of 96/sec. to the same ipsilateral nerve that elicits pure inhibition when stimulated at low frequencies. The series is typical.

The lower limit for the transient reflex is about 60—70/sec.; in some cases an effect can be traced at slightly lower stimulation frequencies. The optimum at about 100/sec. is succeeded by a fall in the transient contraction to be discussed below in relation to additional experimental evidence. If the frequency is only just above the lower limit of the effective range the latent period is lengthened and the contraction reaches its maximal height more slowly. But this secondary reflex is always transitory in the sense that it has disappeared before cessation of the inhibitory stimulus, as can be seen in the records. The removal of the ipsilateral inhibition is followed by a strong contraction involving return of the extensor reflex background to the level kept up by the excitatory stimulus alone. Prolongation of the inhibitory stimulus did not lead to reappearance of the transitory extensor contraction.

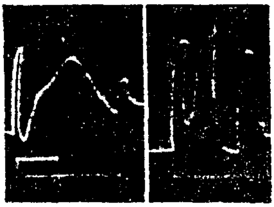

Fig. 2. Crossed and ipsilateral stimulation as in fig. 1. The ipsilateral stimulus at 96 per sec. Two different experiments illustrating extremes of variation in transient reflexes. *Horisontal line in A marks* 20 sec.

Two extremes showing the range within which the type of the transitory reflex has varied are illustrated in fig. 2. The one is a comparatively slow reflex with a drawn-out maximum (2 A), the other one a fast affair with a high maximum (2 B). Quite often clonic contractions are seen to split the contour tracing the transitory contraction, as in fig. 8 A. Some of these variations from case to case may come from the labyrinthine and neck reflexes which influence the transitory contraction just as they affect the extensor reflex itself. Also the level of decerebration is of importance. High sections often lead to slow contractions with clonic movements superimposed (*e. g.* figs. 5 and 8 A), low sections seem to further a transient reflex of the fast type, shown in its most pronounced form in fig. 2.

General Transient Reflex Pattern.

The transient extensor reflex is part of a complex reflex pattern affecting the whole animal. When stimulation of the sciatic at a rate of 90 per sec. has lasted a few seconds the *general reaction* sets in with great force: in parallel with extensor contractions in all limbs the head is drawn towards the trunk, the tail bent in between the

hind legs, and the whole animal assumes a position of ventroflexion. At the maximum of the effect *micturition* is quite common. The whole reflex attitude is transitory, strictly parallel to the extensor contraction and dependent upon frequency of the stimulus just as the latter. At lower frequencies it is less marked and below 50/sec. absent.

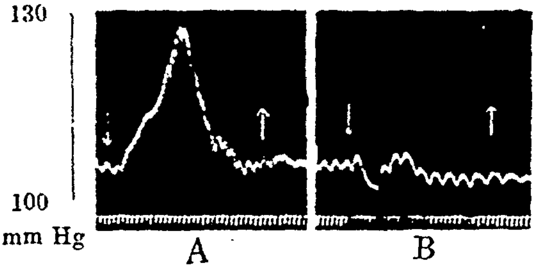

Fig. 3. Blood pressure in *a. carotis* of cat. Stimulation of sciatic nerve between arrows. A at frequency 96, B at 20 per sec. Time in sec.

The occurrence of micturition led to the surmise that other vegetative components might be involved. For this reason *blood pressure* and *pupil width* were recorded in some experiments. The effect on the blood pressure is very marked (cf. AŞCHKENAZ 1939) as shown by fig. 3, the pupil also dilates as illustrated by fig. 4. These effects too are transitory and dependent upon frequency of the stimulus in the same manner as the others. These animals had received dial and did not show the reflex muscular components

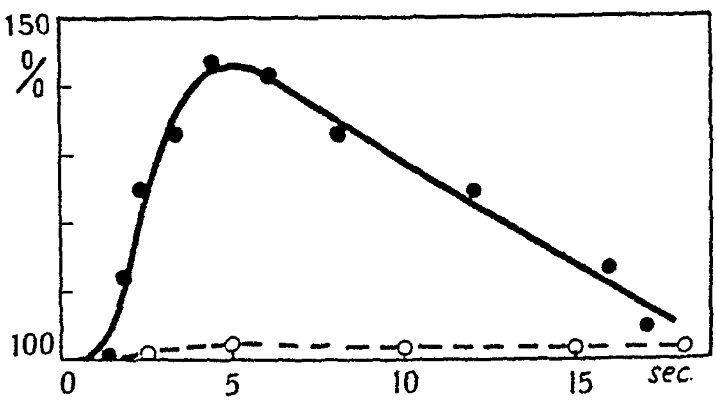

Fig. 4. Change of diameter of pupil in per cent of resting value following stimulation of sciatic nerve at frequencies 90 (line drawn in full) and 20 (broken line) per sec.

of the general transitory effect. Therefore it is clear that the vegetative reactions are not secondary to the motor activity.

The reflex pattern thus seems to be a comprehensive defensor reaction with somatic components combining to the picture of an infuriated cat.

Frequency Variations of Ipsi- and Contralateral Stimuli.

Continuing the analysis with the well defined transient extensor contraction as the best analyzable part of the complex reflex pattern we shall now vary the frequency of the contralateral stimulus eliciting the crossed extensor reflex itself. In fig. 5 A and B there is first the control with an ipsilateral stimulus at 25/sec. inhibiting the crossed extensor (B) but causing the typical transitory extensor contraction at frequency 95/sec. (A). In fig. 5 C, however, the high frequency 95/sec. is used to elicit the same crossed extensor (curve on the left). This now also has a secondary, transitory maximum exactly as in the foregoing case when the ipsilateral nerve was used. But with the frequency 25/sec. (curve on the right) as control there is only the ordinary crossed extensor without any transient secondary reflex. Thus it is unnecessary to elicit the transient effect with the ipsilateral inhibitory stimulus. The contralateral nerve exciting the crossed extensor also causes the same reflex pattern to appear. Frequency, parallelism with the general reflex response, and latent period are identical in both cases. The only difference is that with the ipsilateral inhibitory stimulus the reflex appears as a secondary hump in the trough caused by inhibition, with the contralateral one, as a hump on top of the crossed extensor.

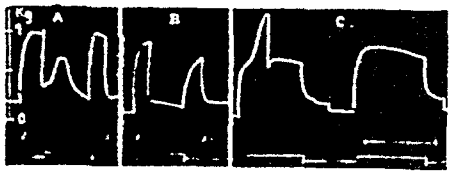

Fig. 5. Reflex contraction of quadriceps muscle. A and B marked as in fig. 1. Ipsilateral stimulation in A at 96, in B at 25 per sec. Contralateral at 25 per sec. in both records. C, contralateral stimulation, first at 96, then at 25 per sec. Horisontal line in C marks 20 sec.

The Stretch-Reflex Background.

Hitherto the crossed extensor, both in the active and in the inhibited state, has been used as background for the transitory reflex. Now with the quadriceps muscle and the ipsilateral sciatic nerve different degrees of stretch-reflex background will be set up by increasing the amount of tension in the muscle. This can be done by raising the myograph on its stand, the cat being clamped to the myograph table and stand with drills through the bones in the usual manner.

In fig. 6 A and B initial tension has been insignificant. At the lower frequency of 25/sec. there is no effect, the higher frequency 95/sec. elicits a typical transient reflex despite the low degree of

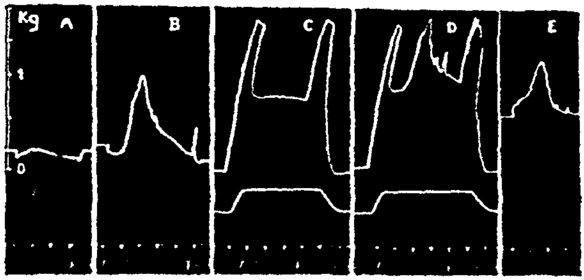

Fig. 6. Reflex contraction of quadriceps muscle. All stimuli ipsilateral. In A and B initial tension approaching zero, frequencies of ipsilateral stimulus respectively 25 and 96 per sec. *In C and D the lower curve illustrates the rise of the myo*graph in order to increase tension. The muscle (above) gives stretch reflex which in C only is inhibited by stimulation at 25 per sec., in D inhibition is succeeded by transient reflex when stimulus frequency has been increased to 96 per sec. E. Same as B, but recorded 1 hour after transection of spinal cord. Time in 5 sec.

stretch (initial tension 0.2 kg.). It is preceded by inhibition of the small stretch reflex under these conditions. The general reaction was also present. In fig. 6 C and D the same experiment is repeated with the muscles stretched to an initial tension (including myotatic reflex) of nearly 2 kg. At the lower frequency (C) there is merely inhibition of the stretch-reflex, at the higher (D) in addition the typical transient extensor reflex accompanied by the other reactions belonging to the same reflex pattern. Thus the transient reflex is little if at all influenced by the myotatic background.

Transient Reflex and Deafferentation.

The results of the previous section indicate that the proprioceptive impulses are of little if any significance for the transient reflex. In order to throw further light upon this aspect of the question some experiments on deafferentation were carried out. On a decerebrated cat, denervated except for the muscle to be studied (*m. quadriceps*), the dorsal roots on the right side were laid bare and from L4 downwards just so many severed that knee jerk and stretch reflex disappeared in the muscle. This was checked by tapping the tendon and pulling on the muscle after the severance of a few strands of fibres at a time. The knee jerk and the stretch-

reflex disappeared when L4, L5 and the greater part of L6 had been severed. The dorsal roots on the left side were intact and stimulation took place through the left sciatic.

Although the reflexly active right quadriceps did not react to a tap on the tendon or to stretch, nevertheless the transient reflex effect was fully developed, as shown by fig. 7 A and B. We may therefore once more underline our previous statement that the proprioceptive impulses from the extensor muscle, thrown into transitory reflex activity by high frequencies of stimulation, are of little if any significance for the latter effect.

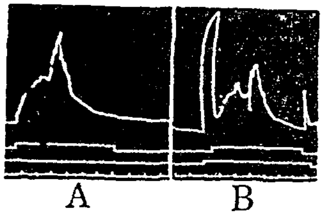

Fig. 7. Reflex contraction of quadriceps muscle after deafferentation (see text). A, ipsilateral stimulation alone at 96 per sec., B, same stimulus against back-ground of crossed extensor elicited by contralateral stimulation at 25 per sec. Time in 5 sec.

Spinal Animals.

The next question concerned the significance of higher spinal segments for the reflex. It is clear from what has been said that it may be modified by segmental activity above the level of the reflex itself, but whether higher centres are essential or not could only be found out by studying spinal animals. The spread of the transitory reflex in so many different directions, particularly its appearance in all legs and the trunk, gave an additional interest to this experiment.

In an animal, the transient extensor reflex of which is shown in fig. 6 A—D, the spinal cord was cut in the region of the last thoracic segments leading to the typical symptoms of spinal shock. After an hour reflex activity was present and the ipsilateral sciatic nerve was now stimulated at different frequencies with the quadriceps muscle attached to the myograph. Fig. 6 E shows that the transitory extensor reflex was readily elicitable by stimuli at a frequency of 95/sec. Thus, despite the presence of a general reaction in the intact animal, this particular component of it has its path laid down in the segments connected to the ipsilateral nerve stimulated.

Frequencies above 100 per Sec.

The experiment of fig. 8 refers to stimulation of the ipsilateral sciatic with the quadriceps muscle attached to the myograph and minimal tension. Three frequencies, 100 (A), 300 (B), and 400 per sec. (C) have been used. The transient reflex diminishes with higher frequencies of stimulation and is absent at 400/sec.

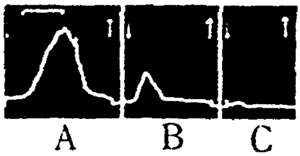

Fig. 8. Reflex contraction of quadriceps muscle. Ipsilateral stimulation between arrows of sciatic nerve at frequencies 100 (A), 300 (B), and 400 (C) per sec. Horisontal line in A marks 20 sec.

A still larger range of frequencies is analyzed in the experiment of fig. 9, where the *frequency variation* is put in over the ipsilateral inhibitory nerve against a background of crossed extension, just as in the experiments presented in the first section. The frequencies range from 20—800 per sec. At the highest frequencies the nerve fibres may alternate and thereby alter the applied frequency. There is first pure inhibition at the low frequencies

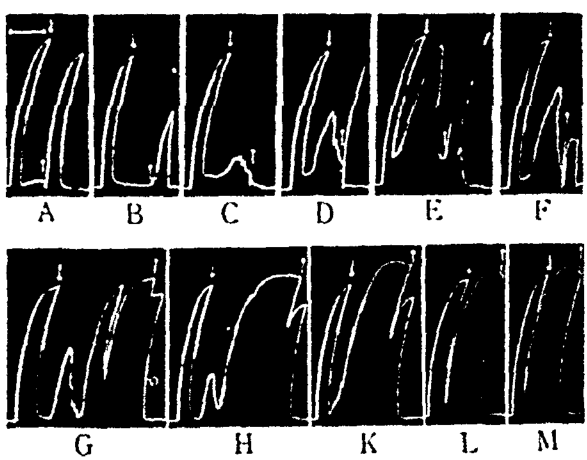

Fig. 9. Reflex contraction of quadriceps muscle. In all records stimulation of contralateral sciatic nerve (not marked) at 25 per sec. leading to crossed extensor. Between arrows stimulation of ipsilateral sciatic nerve at frequencies 20 (A), 60 (B), 70 (C), 80 (D), 90 (E), 100 (F), 150 (G), 200 (H), 300 (K), 500 (L), and 800 (M) per sec. *Horisontal line in A marks 20 sec.*

(A, B); then the transitory reflex sets in at 60/sec. (C), reaches its optimum at 90/sec. (E), thereafter to diminish again at higher frequencies of stimulation. Two complications are visible in the records referring to these frequencies. The first is that the in-

hibitory period shortens and is succeeded by an earlier rise of excitation which follows just after the transient reflex and leads to a higher level of the re-appearing crossed extensor, particularly marked at the three highest frequencies (K, L, M). The second complication consists of an abrupt fall of the extensor contraction when the ipsilateral stimulation stops. This fall seems to have an optimum in record K at 300/sec.

These effects have not been further analyzed and will be taken up separately at some other time.

Different Nerves.

Some less systematic experiments have been carried out in order to find out whether the transitory reflex is restricted to definite input paths. Ipsilateral stimulation of nn. *popliteus*, *peroneus*, *saphenus*, and the hamstring has been used. *N. popliteus* has always been active in our experiments, but the peroneal and saphenous nerve and the hamstring have only elicited inhibition of the crossed extensor, not the transient extensor contraction dependent upon frequency of stimulation.

The Flexion Reflex.

The bilateral extension is the typical transitory effect. As sample of flexor effects was used *m. tib. ant.* reflexly activated at different frequencies through the ipsilateral tibial nerve. Nothing but the typical flexion reflex could be elicited in this experiments. At the higher frequency (96 per sec.) the reflex contraction diminishes after a brief latency. Whether this is due to blocking fatigue or to antagonistic inhibition caused by the transitory reflex in the extensors has not yet been ascertained.

Discussion.

Though it is clear from the results presented that we are dealing with a very characteristic reflex pattern responding optimally to a certain frequency range of stimulation, it is not thereby demonstrated that just this particular reflex is the only one responding optimally to the range in question. We may only have found particularly favourable conditions for demonstrating the effect of frequency.

The limitation of the effect to certain afferents and other facts known about the effect of frequency of stimulation upon peripheral nerve (see *e. g.* GASSER and GRUNDFEST, 1939) would seem to make it highly improbable that the optimal range of frequency is peripherally determined. The mechanism is almost certainly central. Temporal summation may be optimal for the frequency range found and this together with a diminution of inhibition at higher frequencies may be the factors determining the release of the transient reflex under the conditions defined in this paper. But even if this be so, the mechanism must still be said to be unknown. To what an extent reflex excitation generally is favoured by certain rates of stimulation remains to be determined. That it is so in this particular case throws new light upon the significance of the frequency variation of the normal afferent input as a basis of selection.

The transient reflex may be identical with the extensor thrust. Attempts to verify this suggestion have not yet been made.

Summary.

Experiments have been carried out on decerebrate cats with a view to studying the effect of frequency of stimulation upon reflex activity.

With frequencies between 50—100 per sec. stimulation of the sciatic nerve activated a late secondary extensor reflex after a latent period of 3—5 sec. This reflex was transient in the sense that it lasted only about 15—30 sec. The optimal frequency, for which this transient reflex was maximal, was 90—100 per sec.

The transient extensor contraction was found to be part of a complex general defensor pattern involving extension of all limbs, ventroflexion of the trunk and the tail, wihdrawal of the head, and a number of vegetative components such as micturition, raise of blood pressure (cf. ASCHKENAZ 1939), and pupil dilatation. The vegetative effects were also observed in animals which had received dial and therefore did not show the motor reactions. The whole defensor reflex pattern was optimally evoked by the frequency optimal for the extensor contraction.

The transient extensor reflex turned up bilaterally. When evoked by an ipsilateral stimulus in the midst of a crossed extensor it showed itself as a transitory contraction in the trough of the ipsilateral inhibitory effect. However, it turned up equally well

without a background of crossed extension. When elicited by crossed stimulation the transient effect had the shape of a hump on the ordinary crossed extensor reflex, provided that the frequency of the stimulus was sufficiently high.

No proprioceptive background was necessary for the transient extensor contraction, as shown by deafferentation.

Section of the spinal cord in the lower thoracic region did not abolish the reflex.

The transient reflex could be elicited from n. *popliteus* but not from nn. *peroneus, saphenus* and *glut. sup.*

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Transmission of Impulses from Nerve to Muscle Fibre.

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Introduction.

While there is a general agreement concerning the properties of certain chemical substances as mediators of autonomic effects, two opposite views are held as to the transmission of impulses from nerve to voluntary muscle.

According to ECCLES (1936), MONNIER (1934, 1936), et al., the action current accompanying nerve impulses represents the *physiological stimulus and acetylcholine is considered a by-*product of nerve metabolism. BROWN, DALE, FELDBERG (1936) and their collaborators, however, interpret their experimental results as evidence for a chemical transmission and they regard acetylcholine as a mediator of impulses from nerve to voluntary muscle.

In direct measurements, the existence of a polarized interface between nerve and muscle, and its definite relation to the excitability of the muscle could be demonstrated (BUCHTHAL and LINDHARD, 1934, 1935, 1937). Moreover, preliminary reports were given (BUCHTHAL and LINDHARD, 1937, 1939) concerning the stimulating action of acetylcholine when locally applied to the end plate. The present investigation comprises a more detailed study of the stimulating effect of certain chemical substances and their relation to the potential difference between end plate and muscle fibre.

Hitherto nearly all investigations concerning these problems were carried out on whole muscles which were perfused with Ringer solution containing one or more substances, the effect of which had to be investigated. In certain other cases, the perfusion fluid from an active muscle was led through the vessels of another muscle, in order to study whether the first one was forming substances which might cause or at least might be related to muscle contraction. With respect to this technique, a considerable step forward was made by the development of the so-called "close arterial injection" by BROWN, DALE and FELDBERG (1936). The substances were introduced into the afferent artery in the immediate neighbourhood of the muscle. However, these procedures are too summary to give precise information. The dosage is extremely uncertain. Of course, the concentration of the substance in the perfusion fluid or in the blood can be determined, but it remains unknown how large a dose was supplied to the single motor end plate. The distribution of the substance over the muscle with time or space cannot be followed. It cannot be elucidated whether all motor end plates or only part of them are stimulated. Not all stimulated end plates are affected simultaneously, and the time differences are unknown. Therefore, the muscle reaction cannot be estimated even if it is easy to register when whole muscles are concerned. At any rate, we shall get an interference picture of the mechanical reaction which — even if action potentials are led off — will be very difficult or almost impossible to analyze more closely.

These difficulties may be avoided by employing a microtechnique, i. e. by applying the substances, the effects of which have to be investigated, *directly* to the motor end plate. Simultaneously, the reaction of the individual muscle fibre can be observed *directly* under the microscope.

Following a suggestion of Sir HENRY DALE, investigations of the last mentioned type were performed in recent years.

Technique.

As experimental object, muscles of the common lizard (Lacerta agilis) were applied. Frog muscles are less suitable in the present case, due to the special ramification of the motor nerves of Batrachia.[1] However, the use of lizards involves the disadvantage that the animals

[1] This explains KEIL and SICHEL's experiments (1936, 1937).

are available only during the summer months. Even if we succeed in keeping lizards alive throughout the beginning of winter, their muscles react very inertly, and it is impossible to keep isolated fibres alive for a reasonable time. The muscle mainly employed in these experiments and also in other experiments with motor end plates was the profound layer of m. obliquus abdominis internus. The muscle was dissected in such a way that the final preparation consisted of one single layer of muscle fibres and their nerve supply. In some cases, the spinal cord of the preparation was exposed so that the muscle fibres could be stimulated from the corresponding spinal segment. A complete isolation of the single fibre is inappropriate, because an injury of its nerve supply during preparation will often be unavoidable. Furthermore, it was noticed that a completely isolated fibre generally has a shorter lifetime than a fibre which had not undergone so many manipulations. The preparations were kept in oxygen saturated Ringer solution,[1] the pH of which was under control. When not in use, the preparations were kept in a refrigerator or on ice in order to secure a rather low temperature.

The application of stimuli to the motor end plate and the reaction of the muscle fibre were observed under the microscope with a magnification of 150 times. In order to increase the reliability, all observations were performed simultaneously by two observers by means of a double eye piece. When working with preparations containing more than one fibre, this method was preferred to a registration of the mechanical reaction, since it guaranteed that the fibre belonging to the stimulated end plate was reacting. Especially when nerve stimulations are employed, the stimulation would spread to numerous fibres, and sometimes spontaneous contractions of the preparation might occur.

It was shown repeatedly by micro-application of pure Ringer solution that the effects observed were really due to chemical stimulation and not to mechanical stimulation caused by the experimental technique.

The leading-off of *electric potentials* from the muscle fibres was carried out by means of unpolarizable micro-electrodes. Measurements were performed with a d. c.-amplifier and a compensation device (Kelvin bridge). The leading-off electrodes were used also as electrodes for electric stimulation; platinum electrodes were used when the spinal cord was stimulated. Single stimuli (condenser discharges) as well as short-lasting tetanic stimuli (KATZ, 1938) were applied. In order to make sure that the preparation was still alive, a control with direct electric stimulation of the muscle fibre was repeated at suitable time intervals, and especially after conclusion of the experiment. Chemical stimuli were administered on the motor end plate or on the fibre substance, so far as possible in isosmotic solutions, by means of a micro-

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[1] The polyviol Ringer solution was as follows:

6.7 g NaCl	0.2 g glucose	1000 g dest. water
0.2 g KCl	1.35 g Polyviol Am	
0.2 g $CaCl_2$	0.5 g $NaHCO_3$	

An air stream consisting of 1 per cent CO_2 and 99 per cent O_2 was led through this solution. The pH of the solution was then = 7.3.

pipette in connection with an arrangement for quantitative micro-injections. This arrangement and the micro-electrodes were fastened to a Péterfi micro-manipulator. The principle of the injection arrangement, shown in Fig. 1, was similar to that of KROGH's microtonometer. By

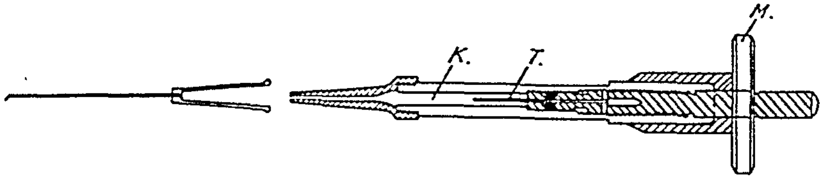

Fig. 1.

the micrometer screw M, the wire T (0.5 mm in diameter) was moved in the chamber K which was filled with paraffine oil. Hereby, the chamber volume was reduced, and a corresponding amount of liquid was pressed out of the pipette. The pipette was treated with paraffine and had an opening of 20—30 μ. The amount of liquid applied to the end plate was 30×10^3 to $102 \times 10^3 \mu^3$. The apparatus was calibrated by weighing. When a less accurate dosage was sufficient, an ordinary "mouth pipette" (Fig. 2) was used. If the diameter of the tip is known, the size of the drop may be estimated with fairly good approximation.

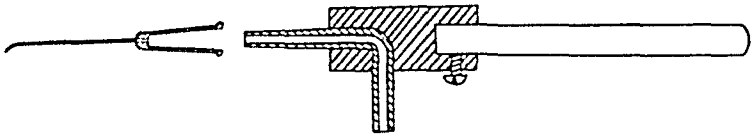

Fig. 2.

As the motor nerve does not end in the fibre substance but in a special organ, the motor end plate, it might be anticipated that the nerve impulse, in order to come from the end of the nerve to the contractile substance, must pass at least two boundary faces, viz. the transition from the nerve to the sole of the motor end plate, and the transition from the latter to the fibre substance. The experiments confirm this assumption, as it has been possible to block the transition of nerve impulses without inhibiting the indirect excitability of the fibre from the end plate.

The following report comprises experiments with chemical stimuli, the effects of which have been discussed in literature in connection with the stimulation process.

Results.

Acetylcholine: As previously mentioned, DALE and his collaborators regard acetylcholine as the substance which normally

transmits the stimulation process from nerve to muscle. If this is so, acetylcholine must be effective in numerous successive doses. It must therefore be presumed that acetylcholine is broken down almost in statu nascendi into ineffective split products. In the case of mammalian and avian muscles, an esterase in the end plate is assumed to hydrolyze acetylcholine to choline and acetic acid. The effect of this esterase may be inhibited by physostigmin or similar substances.

In the case of the lizard muscle, we found that local application of acetylcholine in a dose of about 5×10^{-6} μg to the end plate caused a reaction of the muscle fibre with rapid contraction, similar to a very shortlived tetanus. *Thereafter, all subsequent doses of acetylcholine were ineffective.* Hence, by a minimum dose of acetylcholine applied to the end plates, the skeletal muscle becomes unexcitable, and this effect lasts until the acetylcholine is removed in one way or another. Even the tenfold dose of acetylcholine placed directly on the fibre substance is without any effect; by still larger doses, the fibre is slowly brought to contracture. After washing the preparation in Ringer solution, the fibre responds again to a single dose of acetylcholine and is insensitive to subsequent doses. After one dose of acetylcholine, the motor end plate is blocked for nerve impulses, while the fibre still reacts to electric stimulation of the end plate itself. It has been suggested that the repeated application of acetylcholine is ineffective on account of a contracture following the first application. This assumption can, however, be discarded, since direct observations prove that the fibre does not contract and that it reacts normally towards other stimuli. As might be expected, a preliminary treatment of the preparation with physostigmin is without any influence upon the results of the present experiments. Hence, cholinesterase does not seem to be present in lizard muscle end plates, or if present, it is without functional significance. Supposing acetylcholine to be the normal transmitter of the nerve impulse also in these animals, the reptile muscle must in situ contain an unknown mechanism which gradually removes acetylcholine as it is formed.

As a result of the experiments with acetylcholine, we find that this substance acts as an indirect stimulus of the fibre when applied to the motor end plate. The motor nerve ends with numerous fine loops in the sole of the motor end plate. It must be assumed that the first physiological boundary face directly surrounds

these loops and, therefore, is of a considerable surface — in contrast to the boundary between end plate and muscle fibre. Hence, acetylcholine has the same effect upon the first boundary face independent of whether the substance is applied to one or the other side of this layer. (Fig. 3.) At the same time, as already mentioned, acetylcholine blocks the transmission of nerve impulses as well as the effect of repeated doses of acetylcholine. On the other hand, acetylcholine has no appreciable effect upon the other boundary face, since potassium proves to be effective with unchanged threshold even after several doses of acetylcholine, as will be described below.

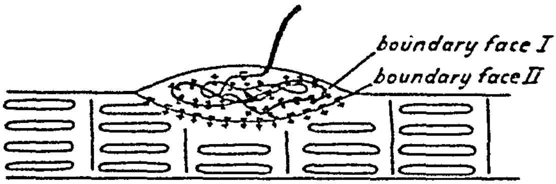

Fig. 3. Diagram of motor end plate showing the boundary faces.

Curarine and *novocaine* show the same effect as acetylcholine upon the first boundary face. However, these substances do not stimulate the muscle fibre. Acting for some time, they also block the second boundary face, and then contraction can only be produced by direct stimulation of the muscle fibre. Curarine thus acts in two stages. The first boundary face of the end plate is blocked rapidly, whereby the muscle fibre becomes refractory to nerve impulses; thereafter, several minutes elapse before an effect can be noticed on the second boundary; as long as the latter is not blocked, an electric stimulation of the end plate will still be effective and "action currents" may be led off from the muscle fibre. These phenomena which can only be observed by direct experiments on end plates are probably the cause of the general uncertainty and the diverging views found in the curare literature. The curare-like effects of acetylcholine were emphasized by numerous authors, among whom also HEIRMAN (1936) who investigated the frog's m. gastrocnemius. This author applied acetylcholine in very large doses and stated that the substance in such doses inhibited the indirect excitability of the muscle. This result may be explained by the fact that HEIRMAN did not stimulate the motor end plate and, therefore, did not consider any other form of indirect stimulation except the nerve impulse.

An interpretation of HEIRMAN's other results is rather difficult, as his technique does not permit a proper analysis of the experimental results.

Another substance which has been brought into relation to stimulation- and contraction processes[1] in the muscle is *potassium* (WILSON and WRIGHT, 1937, and BROWN, 1937), the local application of which would therefore be of some interest.

In contradistinction to the substances previously mentioned, potassium affects the second boundary face of the motor end plate. When applied to the motor end plate, potassium is effective in doses of about 10^{-4} μg (concentration 25—30 mmol) as an indirect stimulus to the muscle fibre. The first, the second, and in general also the third dose produce strong contraction of the muscle fibre. Frequently, however, the effect of the third dose is somewhat reduced. Even in the tenfold concentration, potassium does not stimulate the muscle *fibre* directly; on the contrary, it seems to reduce its excitability. When applied in very large doses directly to the fibre, potassium causes contracture. A blocking of the first boundary face is without any influence upon the potassium effect. After application of acetylcholine, potassium acts with the same intensity as when applied to the fresh end plate, and its threshold value remains unchanged. Potassium further stimulates the end plate which is insensitive to acetylcholine when the nerve impulse is blocked by curarine or novocaine. However, after a long-lasting action of the last mentioned substances, also the second boundary face is blocked, and potassium is now ineffective.

Potassium as well as acetylcholine thus bring about indirect stimulations of the muscle fibre; the potassium effect, however, is not accompanied by a blockade of the transmission from nerve to muscle. After application of potassium to the motor end plate, electric stimuli, and also a first dose of acetylcholine, act upon the nerve or the end plate exactly as in the fresh preparation. A gradual decrease of the potassium effect is not due to blocking of the boundary face, but must be ascribed to other causes. When considering the effect of potassium on the fibre, it must be remembered that large doses of potassium act as a protoplasma poison.

In recent years, the question whether — under physiological conditions — a connection exists between potassium and acetylcholine has produced an extensive literature; nevertheless, the

[1] Cf. FENN's review (1936).

results are not encouraging. The interpretations given by various authors diverge as far as it is possible. This may be ascribed to the fact that the experimental technique in most cases has been inadequate, and the results from these experiments could only permit rather indirect conclusions.

In agreement with our own interpretation, BROWN and FELDBERG (1936) found that the effect of acetylcholine upon the sympathetic ganglions is abolished by curarine, while the potassium effect is uninfluenced. Subsequently, these authors discussed the possibility that potassium ions liberate acetylcholine in the ganglion. According to this view, potassium should act as a nerve stimulus and not as a transmitter.

REGINSTER (1938) emphasized that the nerve impulse liberates acetylcholine which again causes a liberation of potassium ions. Potassium is assumed to be connected with the stimulation process and to be without significance for the muscle contraction itself. This interpretation, however, cannot be maintained. If the effect of acetylcholine consisted in a liberation of potassium ions, it becomes inexplicable that only the first dose of acetylcholine is effective, while potassium still acts after repeated doses, and acetylcholine does not block the potassium effect.

DRUCKREY, HERKEN and BROCK (1939) who worked with liver sections and sections of the salivary gland assumed that a liberation of potassium ions which only occurs during excitation should be regarded as a product of stimulation and not as its cause. Moreover, the liberation of potassium ions should not be a specific acetylcholine effect, since the application of adrenalin and numerous other substances acts in the same way. It is a priori improbable that a strong indirect stimulus such as potassium should appear as a kind of by-product during the stimulation process. As previously mentioned, it is highly improbable that the effect of acetylcholine consists in a liberation of potassium ions. Finally, experiments with local application of adrenalin indicate that this substance does not affect the motor end plates. Therefore, it is not reasonable to assume that adrenalin liberates potassium ions.

According to CICARDO and MOGLIA (1940), a liberation of potassium ions proportional to the acetylcholine concentration occurs after intravenous injection of acetylcholine in a potassium-free Ringer solution. These authors emphasize that the significance of acetylcholine for the contraction process is based upon a sub-

stance "composé organo-potassique", liberating potassium which is regarded as the true cause of muscle contraction. There is hardly any reason to discuss this theory more thoroughly. It has repeatedly been pointed out why potassium cannot be considered as the proper "contraction substance"; as a further argument may serve the observation that a longer lasting action of potassium injures the muscle fibre. Hence, the above theory claims the existence of a substance which continuously absorbs potassium ions in the course of their formation.

In order to investigate whether the potassium effect observed is specific or whether related substances produce the same or similar effects, the influence of *rubidium* has been studied in a series of experiments. It was found that equimolar concentrations of rubidium in every respect behave like potassium. On the other hand, both *sodium* and *calcium* in corresponding or considerably higher concentrations have been without noticeable effect upon the motor end plate.

Though *veratrine* does not act as a stimulus to the end plate, its influence on transmission and contraction is of great interest. To veratrine certain sensitizing properties have been attributed, because a veratrine-poisoned muscle reacts to a single impulse with a strong, protracted contraction. This phenomenon has been observed in a number of experiments on the isolated fibre. A veratrine-poisoned preparation responds to the nerve impulse and to stimulation of the motor end plate with electric stimuli, acetylcholine and potassium with protracted contractions. Our experiments do not reveal the cause of this peculiar effect. A "sensitization" in the usual sense is improbable, as this would lead to reduced thresholds for the respective stimuli. In the veratrine-poisoned preparation, the acetylcholine threshold as well as that of potassium were the same as in a fresh preparation. Also in cases where veratrine was given together with curarine, potassium applied to the end plate produced protracted contractions as long as the second boundary face of the end plate was not blocked. If veratrine was supplied together with *quinine*, however, the contractions were not protracted. In our experiments, application of quinine to the motor end plate had no appreciable effect. Also novocaine suppressed or abolished the action of veratrine. In this respect, veratrine poisoning reminds of *myotonia*. The myotonic symptoms disappear in the course of a few minutes after injection of novocaine or quinine. These observations indicate

that the myotonic reaction is initiated from the motor end plate (BUCHTHAL and CLEMMESEN, 1942). It is worth mentioning that the veratrine-poisoned preparations — in contrast to all other cases investigated — responded to repeated doses of acetylcholine. This might indicate that the veratrine effect is analogous to that generally ascribed to the cholinesterase, viz. a rapid disorganisation of the liberated or applied acetylcholine.

HARVEY (1939) observed a reducing effect of quinine upon the excitability of the motor end plate, quinine suppressing the effect of veratrine and eserin. Furthermore, HARVEY stated that quinine counteracts the effect of acetylcholine, while the action of potassium is only slightly influenced. HARVEY worked on mammalian muscle, and this may explain why his results deviate from ours. As previously mentioned, however, a marked effect of acetylcholine in threshold doses was obtained after treatment of the preparation with quinine.

SZENT-GYÖRGYI, BACQ and GOFFART (1939) perfused a frog muscle with veratrine Ringer solution and then led the perfusion fluid through another muscle. If muscle I was stimulated with a tetanizing stimulus, muscle II contracted. The authors pointed out that this contraction cannot be ascribed to acetylcholine, since muscle II would also contract if muscle I was curarized. However, from the fact that curarine blocks the end plate with respect to acetylcholine, it cannot be concluded that curarine inhibits the liberation of this substance. It is furthermore improbable that the contraction of muscle II is due to potassium ions, because veratrine does not sensitize the muscle with respect to potassium, but only leads to protracted contractions.

A scheme of the experimental results already discussed is given in Table I.

Formerly, an attempt has been made to follow the stimulation process from nerve to muscle fibre by a registration of the variations in potential difference between the end plate and the fibre substance ("action potential"). Immediately after electric stimulation of the end plate, a sudden decrease in the potential difference was found which returned rapidly to the initial value. The total variation occurred within 2—3 msec. When the motor end plate was poisoned with curarine, the action potential decreased — frequently after a slight initial increase — to zero in the course of 10—30 minutes, and the end plate must then be regarded as an inactive continuation of the micro-electrode. The muscle fibre was then

Table 1.

After treatment of the end plate with	Stimulation of nerve	Stimulation of end plate with		
		electr. stim.	acetylcholine	potassium
0	+	+	+ — —[1]	+ + (+)[2]
Acetylcholine	—	+	— —	+ + (+)
Potassium	+ (+)	+ (+)	+ — —	+ (+)
Curarine, short duration . . .	—	+	— — —	+ + (+)
Curarine, longer duration . .	—	—	— — —	— — —
Novocaine, short duration . .	—	(+)	— — —	+ — —
Novocaine, longer duration . .	—	—	— — —	— — —
Veratrine	+ pr.[3]	+ pr.	+ (+)(+) pr. pr. pr.	+ + + pr. pr. pr.
Quinine	+	+	+	+
Novocaine + veratrine	—	—	—	—
Quinine + veratrine	+	+	+ (+)(+) not pr.	+ + (+) not pr.

refractory to indirect stimulations. However, these alterations were reversible; as soon as curarine was removed, the potential difference increased to its initial value, and the excitability to indirect stimuli was regained. The same effect was obtained by irradiation with radium emanation, however, the latter effect going still further, since also the contraction potential was abolished and the fibre became irresponsive to direct stimulation. In numerous cases, these alterations were also found to be reversible even after long-lasting irradiation.

When the motor end plate was stimulated with acetylcholine, a decrease in the potential difference between end plate and muscle fibre was found, which generally reached its maximum in the course of 10 sec. In all cases, the potential increased again rather rapidly, this increase was, however, slower than the initial decrease. The potential did not always reach its initial value during the time of observation (6—8 min.).

The potential variations showed a completely corresponding course after application of potassium or rubidium. They always

[1] Effect of 3 successive doses.
[2] (+) indicates a somewhat weaker reaction.
[3] pr. = protracted contraction.

appeared when the respective substance was applied to the end plate, but the decrease in potential did not always occur at the same rate, and the size of the deflections varied.

That the potential oscillations after chemical stimulation are considerably slower than after electric stimuli may be explained by the assumption that the chemical stimuli partly are less effective, partly undoubtedly have a tetanizing action. From this, it may be concluded that the observed decrease in potential is composed of a number of small successive potential alterations. But even if each stimulus gives rise to a decrease in the potential difference, this effect must not necessarily be followed by a contraction; the variations can either be too small or they can occur too slowly. Therefore curarine, for instance, does not act as a stimulus, although this substance completely abolishes the potential difference.

As long as the decrease in potential is not compensated, the muscle fibre must be refractory to indirect stimulation. Perhaps, this persisting reduction in potential is the reason why only the first dose of acetylcholine is effective or, rather, why doses following immediately after the first one are ineffective. Corresponding considerations might possibly be applied to the previously discussed decreasing effect of repeated doses of potassium.

Summary.

Direct application of different substances to the motor end plate of lizard muscle led to the following results:

Acetylcholine (threshold 5×10^{-6} μg) initiates a short twitch-like contraction. After the first application, the end plate is refractory to further doses and to electric stimulation of the nerve.

Direct electric stimulation of the end plate and application of *potassium* (threshold 10^{-4} μg) or *rubidium* are still effective. Potassium or rubidium do not show any blocking action, as does acetylcholine.

Acetylcholine and potassium applied to the *fibre substance* have no effect even in a tenfold concentration.

Acetylcholine, potassium, and other stimulating substances cause a decrease in the end plate — fibre potential.

The threshold of the motor end plate is not altered by poisoning with *veratrine*. Just as total muscle, the veratrinized fibre reacts to stimulation with protracted contractions.

In the first stage, *curarine* blocks the end plate for nerve impulses and for acetylcholine, while direct electric stimulation of the end plate and application of potassium are still effective. In a later stage, the excitability in response to these stimuli is abolished, only the direct excitability of the fibre substance is retained.

The present material supports the assumption of two boundary aces in the motor end plate.

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From the Zoophysiological Laboratory, University of Copenhagen.

The measurement of the Peripheral Blood Flow by Means of Plethysmography and Skin-Temperature Determinations.

By

E. HOHWÜ CHRISTENSEN and MARIUS NIELSEN.

(Recieved 27 April 1942.)

In preparation for a study of the circulation in the skin during varied external temperature and transition from rest to work, certain methods, considered suitable for giving information about the blood flow and blood content of the skin have been investigated and compared.

In 1938 and 1939 BURTON suggested a method for the measurement of the blood flow in the skin and a similar method was published 1938 by WILKINS, DOUPE and NEWMAN. The principle of this method is to cut off the "venous return" in the finger and plethysmographically register the simultaneous arterial inflow. BURTON states moreover in the same paper, that in the amplitude of the pulse volume one has a relative measurement of the blood flow, as in his own investigations he observed a close correlation between these two functions. We have now submitted BURTON's methods of measuring the skin's blood flow, to a closer investigation of their adaptability for the solution of our problems.

That the finger is regarded as especially suitable for the measurement of the skin's blood flow is due as emphasized by BURTON particularly to the fact that the vascular tissue can be looked upon as being almost exclusively skin tissue.

For the same reason, temperature measurements on the fingers are especially suitable for information concerning changes in the skin's blood flow. A variation in the flow will very rapidly appear

as a corresponding temperature change partly due to the finger's insignificant heat capacity, and partly because of the very slight heat production. In our experiments, the temperature measurements were executed by means of the thermoelement described by one of us (NIELSEN 1939) which is especially suited for this purpose.

Description of the Applied Method.

For the determination of the blood flow using BURTON's technique the finger is placed in a well fitting plethysmograph of aluminium. The plethysmograph is made air-tight around the finger by means of gauze strips impregnated with zinc oxide in vaseline, and through a thick-walled rubber tube its air volume is connected to an exceedingly sensitive tambour. The membrane of the latter is equipped on one side with a small mirror whose deflection is photographically registered. The deflection of the mirror is calibrated by means of a 0.1 cc syringe pipette. The maximum deflection that can be registered corresponded in our experiments to 3 × 0.1 cc and is in its entire length proportional to the volume change. The calibration was constant for the same membrane from day to day, and 0.1 cc corresponded very closely to a deflection of 1 cm on the photographic paper.

The "venous return" from the finger was cut off by means of a small pneumatic cuff about 1 cm wide placed proximately on the finger. Compression was instantaneous, when the cuff was connected to a 5 L. container pumped up to the required pressure.

The Importance of the Filling up Conditions for the Blood Flow Measurement.

In order that the volume increase recorded shall be a direct measurement of the normal inflow into the finger, the flow must take place against a uniform resistance — the volume increase must not be accompanied by any rise of pressure. As long as this condition is fulfilled, the volume curve will be a straight line (see figure 1 a), and the magnitude of the blood flow can be exactly determined. The greater the blood flow into the finger the more rapidly a counter-pressure against the flow will arise, — which brings about a deviation of the curve recorded (see figure 1 b). With a sufficiently rapid blood flow the refill curve will deviate already from the moment of compression (see figure 1 c). In this case, according to BURTON, a measurement of the flow is obtained

by drawing a tangent to the curve through the starting point. It will however always be difficult to place this tangent accurately as the refill curve is not very well defined, especially if the inflow conditions change essentially already during the first stroke of the pulse. Furthermore, the drawing of the tangent is often rendered difficult, because at the time of compression a certain amount of blood and tissue is pressed into the plethysmograph as a result of the pressure increase in the cuff, where by a displacement of the curve can occur.

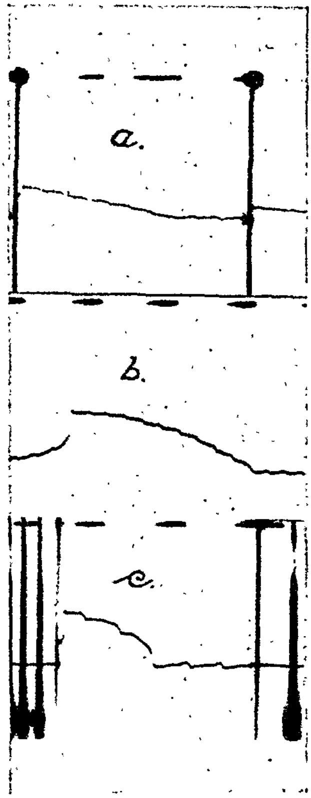

Fig. 1. Examples of blood refill curves with
a) small blood flow
b) medium » »
c) very large » »

The better the refilling conditions, the better the possibility of obtaining linear curves, even during a relatively large inflow which, as is evident from the above mentioned, insures the accuracy of the determination.

In order that the conditions for refilling can be satisfactory it is of course assumed that the plethysmograph is fitted air-tight round the finger without obstructing the "venous return" as venous stasis will rapidly complicate the inflow.

As the refilling occurs mainly in the veins it will be especially the state of filling and tonus of these that is of decisive importance. It could therefore be supposed that an elevation of the hand above heart level and the consequent evacuation of the veins, might greatly improve the refill conditions, and thus produce increased accuracy of measurement. One difficulty encountered with this procedure is that the blood flow can possibly be influenced by the change in hydrostatic pressure in the finger's arteries. One cannot know beforehand whether an adequate blood flow is in such circumstances brought about by compensatory dilatation of the afferent vessels. When comparing blood flow determinations during rest and work where the arterial blood pressure can become very high such a hydrostatic influence might fatally influence the result.

Table 1.

Cuff pressure mm Hg	At heart level			At 40 cm. above heart level		
	Refilling volume cc	Flow cc/min.	Pulse volume cc	Refilling volume cc	Flow cc/min.	Pulse volume cc
30	0.05	2.8	0.015	0.10	2.7	0.026
40	0.075	3.1	0.016	0.13	2.5	0.027
50	0.09	3.8	0.016	0.16	2.6	0.026
60	0.10	3.7	0.016	0.17	3.4	0.023
70	0.105	3.1	0.013	0.18	2.7	0.027

In order to investigate the dependence of the "filling up" conditions on the position of the hand in relation to heart level, experiments have been made both with the hand at heart level and with the hand raised 40 cm. In the first case, the hand rested on the table at heart level, in the second the elbow rested on the same table and the hand was raised 40 cm, by flexion of the elbow joint, and kept lightly supported. The cuff pressure applied to cut off the venous flow, varied, as shown in Table 1, between 30 and 70 mm Hg. Figures 2 a and 2 b show the photographic record of "filling up" in an experiment with the hand at heart level and with the hand raised 40 cm. As seen from Fig. 2 a and 2 b and Table 1, where the results are average values from four measurements at each pressure, the "filling up" is considerably larger in the experiments with the hand raised, and consequently the blood flow can be measured with greater accuracy. The blood flow measured in the two positions shows that the reduced hydrostatic arterial pressure in the 40 cm

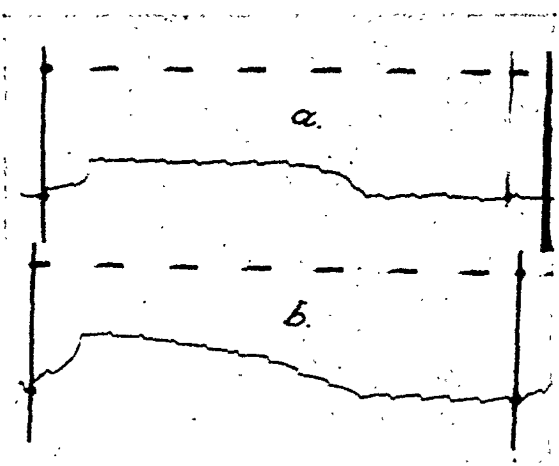

Fig. 2. Blood refilling curves
a) the hand at heart level
b) » » raised 40 cm

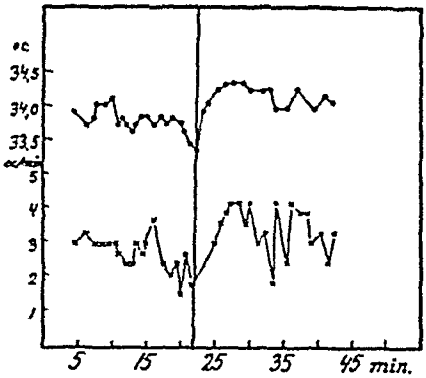

Fig. 3. Subject E. H. C.

Experiments with the hand raised 40 cm above heart level and with the hand at heart level.

Blood flow × and finger temperature ●.

At the perpendicular line the hand is lowered from the 40 cm position to heart level.

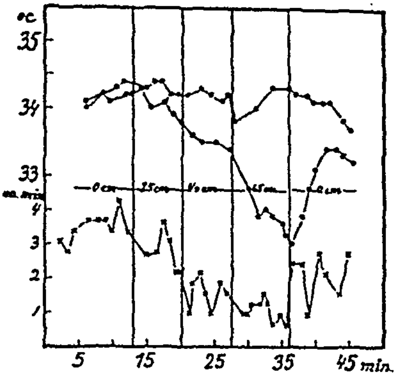

Fig. 4. Subject E. H. C.

Experiment with the left hand at heart level (0 cm) and raised 25 cm 40 cm 65 cm above heart level.

× the blood flow — left hand
○ finger temperature — left hand
● finger temperature — right hand (control at heart level)

position, has not been completely compensated by vasodilatation. In Fig. 3 also, single measurements of the blood flow are graphically represented, indicating a reduced flow in the 40 cm position. This is also evident from the temperature curve in Fig. 3, which shows a slightly lower finger temperature in this position. The temperature is measured with the already mentioned thermoelement placed on the neighbouring finger. As the conditions for refilling are essentially improved while the hydrostatic reduction of the blood flow is insignificant, *we recommend as the normal measuring position, one in which the hand is raised 20 to 30 cm above heart level.*

Fig. 4 shows results of an experiment where the position of the hand is varied between heart level and an elevation of 25, 40 and 65 cm respectively. Blood flow measurements show a reduced flow in the raised positions of the hand, especially at 65 cm. In the highest position the plethysmographic blood flow measurements are somewhat unreliable, as the arterial blood pressure in the finger is so slight that one can only with difficulty obtain a complete cessation of the "venous return" without simultaneously reducing the arterial inflow. The subject of the experiment in question had a blood pressure of 100/60 measured at heart level. When the finger is raised 65 cm the arterial pressure in the finger is therefore reduced to about 50/10, so that the required cuff

pressure may easily exceed the arterial pressure in the finger. In these experiments the skin temperature was also measured on the neighbouring finger. The temperature of the finger shows a gradual reduction in the 25, 40 and 65 cm positions. In the 25 cm position the fall in temperature is, however, very slight, which indicates an almost complete compensation for the altered hydrostatic pressure. On the other hand, in the 65 cm position, the temperature decrease is very considerable indicating that corresponding compensatory dilatation of the vessels has not occurred. In lowering the hand to heart level the temperature curve shows a steep increase although not attaining the original level which is in accordance with the result of the blood flow measurement. This lower level coincides with a more extensive reduction of the skin temperature which is seen in the control curve obtained by temperature measurements carried out on the corresponding finger of the other hand. This hand was kept at heart level during the entire experiment.

In such cases where the blood flow measurements are for various reasons rendered difficult, the temperature measurements on the next finger is a valuable help in the evaluation of results of the flow measurements. However, the temperature can never reflect the rapid variations which can be determined by means of blood flow measurements, even if the finger's thermal lag is, as previously mentioned, comparatively slight.

In addition to the above mentioned experiments with the hand above heart level, a few experiments were made with the hand below heart level. In these experiments, practically no filling up of blood occurred as a result of compression of the veins, thus rendering blood flow measurements impossible. The simultaneous measurement of the finger temperature showed an insignificant rise corresponding to a slight increase of blood flow in this position.

Investigation of the Cuff Pressure Employed in the Measurement of the Blood Flow.

The condition enabling the refill curve to be an expression of the blood flow is that the cuff pressure is high enough to completely arrest the "venous return" for the time being. At the same time, the pressure must not be so high that it affects the arterial inflow, viz. it must be smaller than the diastolic pressure in the

finger artery. As is seen in Table 1 a complete venous barrier is established in these experiments at a pressure of about 40 mm Hg, while a pressure of 30 mm Hg in the pneumatic cuff has evidently been insufficient in the experiments with the hand at heart level. Pressures of 70 mm Hg appear to have practically permitted complete arterial inflow, also in the experiments carried out in the 40 cm position. In numerous experiments made at heart level, we have observed apparently unchanged inflow with a compression of 80 to 90 mm Hg. The subjects we have investigated have generally had a blood pressure of 100/50 or 60. The cuff pressures of 80 to 90 mm Hg are therefore considerably above the subject's arterial diastolic pressure as measured in the usual manner by auscultation on the arm and it appears paradoxical that the arterial inflow could remain undisturbed. We have therefore undertaken a closer investigation of the effect of the pressure in the finger cuff as compared with the pressure in the arm cuff applied to determine the arterial pressure.

For this purpose, the arterial pressure is varied by letting the subject perform work of different intensities. The measurement of the arterial pressure was made in the usual way by auscultation on the arm at heart level. The arterial systolic pressure in the finger was defined at the pressure necessary just to prevent arterial inflow in the finger as recorded by the plethysomgraph. The two pneumatic cuffs were both placed at heart level. The results of such a series of experiments carried out on the subject most frequently used E. H. C., are reproduced in Fig. 5. Determinations were made on the subject at rest and during work, varied between

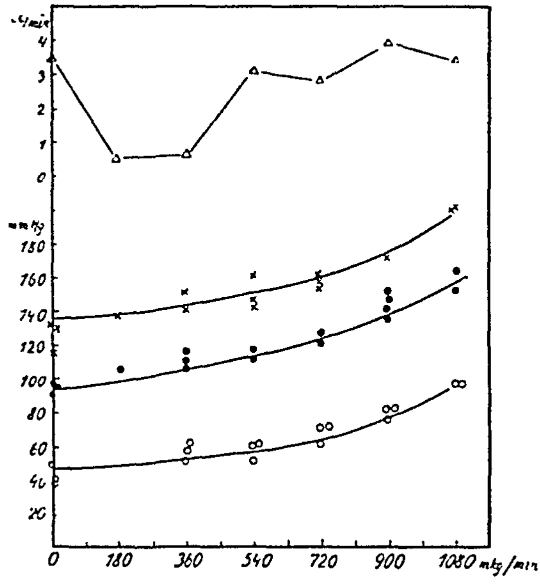

Fig. 5. Subject E. H. C.

△ blood flow
× finger cuff pressure
● systolic pressure
○ pulse pressure

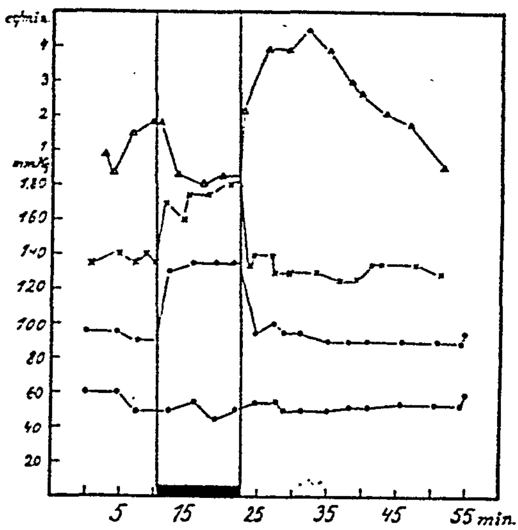

Fig. 6. Subject E. H. C.
Work experiment of 900 mkg/min.
△ blood flow
× finger cuff pressure
● systolic pressure
○ diastolic pressure.
The work period is marked ▬▬

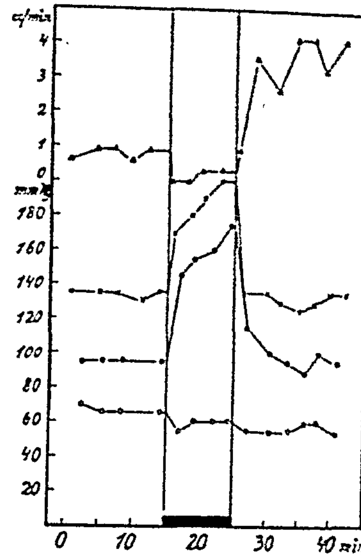

Fig. 7. Subject E. H. C.
Work experiment of 1 080 mkg/min.
△ blood flow
× finger cuff pressure
● systolic pressure
○ diastolic pressure
The work period is marked ▬▬

180 and 1080 mkg/min. The systolic blood pressure hereby varied from about 95 mm Hg at rest to about 160 mm during the heaviest work. The simultaneously measured pressure in the finger cuff varied between about 135 at rest to about 200 during the heaviest work, and as is evident from the figure the curves in their entire extension were practically equidistant. The finger cuff pressure was therefore in these experiments systematically about 40 mmg Hg above the systolic pressure measured on the arm. The diastolic pressure measured in the brachial artery was both at rest and during work about 50 mm Hg. Fig. 6 shows individual determinations from a work experiment of 900 mkg/min. on the same subject. At rest as well as during exertion and the subsequent restitution period, the curves for the systolic arm and finger pressure progressed practically in parallel with a distance between them corresponding to a pressure difference of 40 mm Hg. The diastolic pressure in the brachial artery remained roughly at 50 mm Hg. Fig. 7 shows a corresponding experiment with work of 1 080 mkg/min. This work is at the upper limit of what the subject

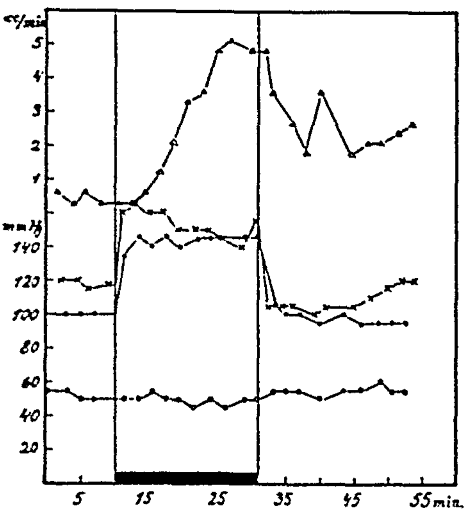

Fig. 8. Subject M. N.
Work experiment of 900 mkg/min.
△ blood flow
× finger cuff pressure
● systolic pressure
○ diastolic pressure
The work period is marked ▬

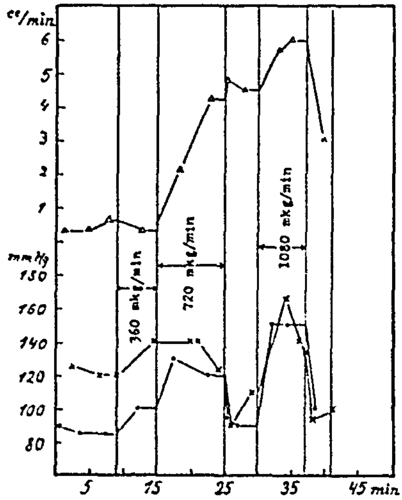

Fig. 9. Subject B. H.
Work experiment of 360, 720, 1 080 mkg/min.
△ blood flow
× finger cuff pressure
● systolic pressure

can perform and we observe here a continued increase of the systolic pressures during the work.

The systolic compression pressure measured by the means of the finger cuff is therefore, in the subject E. H. C. generally approx. 40 mm Hg above the systolic compression pressure measured by means of the arm cuff. It is therefore assumed that the pressure required to exceed the diastolic pressure must also be about 40 mm Hg higher in the finger cuff than in the arm cuff. A compression pressure of 80 to 90 mm Hg in the finger cuff therefore corresponds in this subject to a pressure of only 40—50 mm Hg in the arm cuff, and hence the explanation why these high pressures can be exerted without preventing complete arterial inflow.

In contradistinction to the subject E. H. C. who usually exhibited a constant difference between compression pressures in the finger cuff and in the arm cuff, we found that several of the other subjects showed in the course of a work period a decreasing finger cuff pressure accompanying the increase in the finger's blood circulation so that the pressures in the two cuffs approached and frequently became practically identical. In Fig. 8 and Fig. 9 one sees for example, how the two pressures at the moment when the

flow is highest, viz. at the termination of the period of exertion and the commencement of restitution, coincide, while there was otherwise a difference of about 20 to 40 mm Hg between the two pressures.

We have seen that the compression pressure in the finger cuff in the subject E. H. C. was constantly about 40 mm Hg above the pressure in the arm cuff in spite of the fact that the flow pressure must necessarily be lowest in the finger. As factors that can conceivably influence the systolic compression pressure as measured the following can be enumerated viz. the anatomical position of the arteries, the mass of tissue between the pressure cuff and the artery and even the compressibility of the arterial walls and probably also purely technical conditions such as the width and resistance of the pressure cuff. In addition, it has been maintained by various authors that compression should be dependent to a not insignificant extent on the volume flow of blood so that an increase of the flow should be accompanied by rise in the compression pressure. As regards the latter point the experiments on E. H. C. (see Figs. 5, 6 and 7) show that even large variations in the blood flow are without any significance for the measured compression pressure. In the case of the subject E. H. C. one cannot decide a priori which of the remaining factors may cause the considerable pressure difference between the two compression pressures. With regard to the other subjects, however, the fall that occurs in the finger compression pressure when it approaches that of the arm and the blood flow is maximal (compare for example Fig. 8 and Fig. 9) may be explained by variations in the compressibility of the main arteries of the finger as all the other factors enumerated must be regarded as unchanged during the entire experiment. As the finger cuff pressure with a very large blood flow can become equal to or even lower than the arm cuff pressure, one must assume that the whole difference observed between the two pressures when the flow is slight, must in these subjects be due to a high resistance against compression in the finger arteries. It is a reasonable assumption that such small arteries as those in the finger must, in a state of partial contraction, offer a relatively high resistance towards compression. It must be supposed that the considerable difference between the two pressures in the case of subject E. H. C. must also be attributed at least mainly to a low compressibility of the wall of the finger arteries. When the compression pressure of the finger arteries in

subject E. H. C. is not appreciably affected by variations in the blood flow, it may possibly be explained by assuming that in this subject during a large blood flow a less pronounced dilatation of the main finger arteries occurs than in the other subjects, while the increased flow is in this subject brought about practically exclusively by dilatation of the arterioles.

As is evident from the above there are certain difficulties in the way of deciding in advance the cuff pressure that should be applied for measuring the blood flow, as the highest limit for that pressure allowing complete arterial *inflow* varies considerably in different subjects, and even in the same subject it can vary within wide limits possibly as a result of different compressibility of the finger artery. It is therefore advisable to make a systematic investigation of each subject in order to establish which cuff pressure will give a constant blood flow under the particular conditions. This can be done by varying systematically the pressure in the cuff and making 4—5 measurements at each pressure, as shown in Table 1. For the daily routine a pressure should then be selected from the lower portion of the pressure range found, so that an altered compressibility of the artery does not affect the results of measurements.

The Pulse Volume as an Expression of the Rate of Blood Flow at Rest and During Work.

In the before mentioned work of BURTON 1939, it is stated that for a detailed analysis of the rapid changes in blood flow one can with advantage utilize the uncompressed pulse volume as an expression of the blood flow, as in the investigated cases a close correlation was found between these two factors. We have now investigated in a series of experiments, whether, from a determination of the pulse volume one can obtain an expression for those changes taking place in the blood flow as a result of muscular activity. Fig. 10 gives the results of such an experiment: As seen from the figure there can, under reasonably uniform conditions, for example during the two periods of work, exist satisfactory conformity between the variations in the magnitude of the pulse volume and the blood flow. On the other hand, if we compare the respective values in the period of recovery after the heavy work (1 440 mkg/min. and 720 mkg/min.) one observes a steady fall in

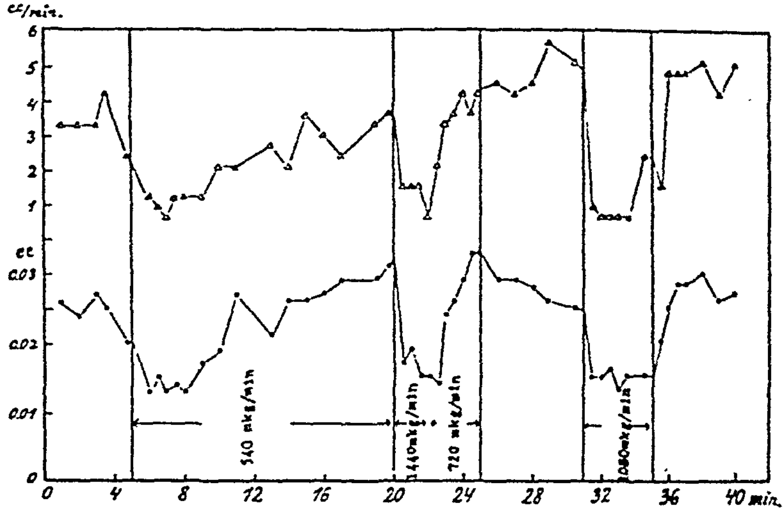

Fig. 10. Subject E. H. C.
△ blood flow
● pulse volume

the pulse volume whilst the blood flow curve indicates a considerable increase. The cause for the diminution in the pulse volume in spite of the increase in the blood flow, is presumably the heavy fall in the pulse pressure during this period. Also during the period of recovery from the work of 1 080 mkg/min. the pulse volume is relatively small in proportion to the very large blood flow compared for example with the values during work of 540 mkg/min., where with the same pulse volume one has a smaller blood flow. On the basis of these experiments we do not consider it possible to utilize the pulse volume as an expression of the blood flow, in connection with muscular activity where large blood pressure changes can appear. As may be expected, the method is also unsuitable in such cases where considerable hydrostatic changes in the blood pressure occur, as seen in Table 1 page 152.

Summary.

In the present paper we have submitted to closer investigation, the methods given by BURTON for the measurement of the skin's blood flow by means of finger plethysmography and the results are compared with skin-temperature determinations.

Our experiments show that the cuff pressure necessary to arrest the venous return, varies greatly from one subject to another and

also in the same subject under different conditions. The reasons for this have been submitted to closer analysis and as the essential reason for this variation is put forward a varied compressibility of the finger arteries corresponding to a varied degree of contraction.

It could be shown that the refill curves recorded photographically allow a far more accurate determination of the rate of flow if during measurements the hand is placed 20—30 cm above heart level; whereby the blood volume, that can flow into the veins in the course of the registration period without pressure increase, rises very considerably.

If the hand is raised above heart level a reduction of the blood flow occurs as the reduced hydrostatic blood pressure in the finger artery is not entirely compensated by means of vasodilatation. With a rise of 20—30 cm, which is here recommended as the normal measuring position, this reduction is only slight however.

Where considerable changes occur in the blood pressure as in the transition from rest to work or vice versa we find it impossible to utilize the finger's pulse volume as an expression of its blood flow.

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From the Laboratory of Zoophysiology, Copenhagen University.

Investigations of the Circulation in the Skin at the Beginning of Muscular Work.

By

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assisted by BIRGER HANNISDAHL.

(Received 27 April 1942.)

It is a general assumption that collateral vasoconstriction and emptying of blood depots occur at the beginning of muscular work in order to provide the possibility of an increased cardiac output together with the maintenance of a constant arterial blood pressure (compare KROGH, 1912). The purpose of this work is to determine if the skin participates in these functions.

Most of the experiments were made on the subject's fingers, where the vascularised tissue is almost exclusively skin tissue and where the quantitative variations must be expected to be especially pronounced due to the arterio-venous anastomoses. The direct blood flow measurements were made by means of BURTON's plethysmographic method, by which the arterial inflow is measured, while the venous return is cut off for a few seconds. For a more detailed description we refer to an earlier paper (HOHWÜ CHRISTENSEN and MARIUS NIELSEN, 1942). Furthermore the changes in the skin circulation in the fingers and other skin areas were studied by means of skin temperature measurements. For these measurements the skin thermocouple described by NIELSEN (1940) was used. All the work experiments were carried out on KROGH's bicycle ergometer.

Fig. 1 shows the result of blood flow measurements on the subject B. H. during work of intensities between 360 and 1 260 mkg/min. In all cases an instantaneous reduction of the blood flow

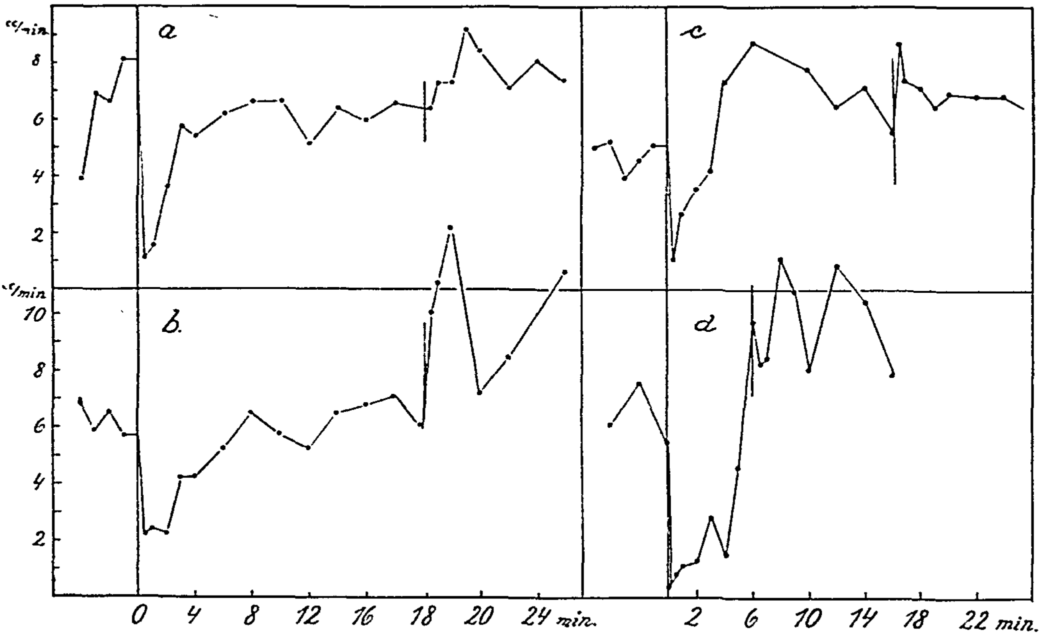

Fig. 1. Blood flow in index finger.

Subject B. H.	a	360	mkg/min.	18.0—18.3° C.
	b	720	»	21.8—23.0° C.
	c	1 080	»	19.0—19.7° C.
	d	1 260	»	18.0° C.

The work begins at zero minute, and the stop is marked by a short vertical line.

occurs at the beginning of exertion; later in the working period the blood flow increases again and in many cases exceeds the preceeding resting value. During the heaviest work of 1 260 mkg/min. which approaches the subject's working capacity, the largest and most prolonged reduction in the blood flow is observed.

Fig. 2 shows corresponding measurements on the subject M. N. during work of 720 mkg/min. Here also a transitory reduction of the blood flow is found during the initial working minutes.

Fig. 3 arises from an experiment with subject E. H. C. The intensity of work was here 1 080 mkg/min. The two short periods of exertion are separated by an interval of 10 minutes. In both work periods the flow measurements show a considerable reduction of the blood flow at the beginning of exertion. In this experiment the skin temperature was measured by means of a skin thermocouple pasted on the dorsal side of the neighbouring finger. In accordance with the blood flow measurement the simultaneously registered skin temperature shows a decrease at the beginning of work. The

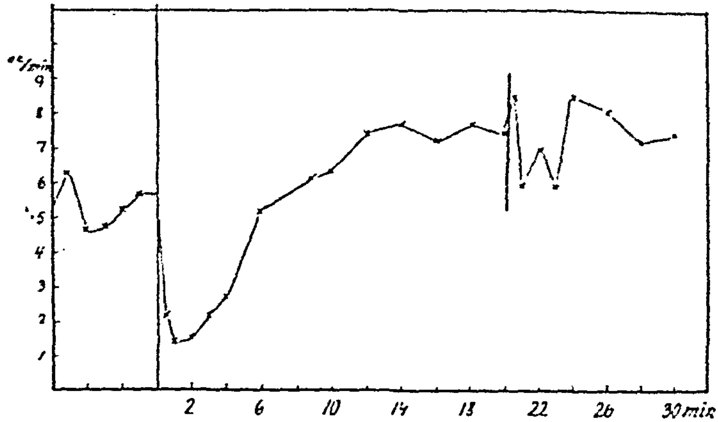

Fig. 2. Subject M. N. 720 mkg/min. 22.1° C.

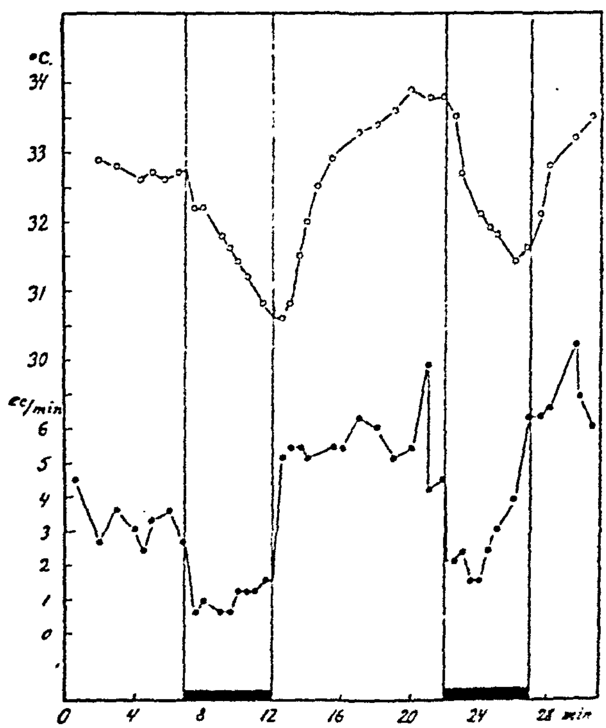

Fig. 3. Subject E. H. C. 1 080 mkg/min. 22.2° C.

● Blood flow in index finger
○ Temperature of third finger

The two work periods are marked ▬

more gradual reduction of the *finger* temperature must be attributed to the thermal lag.

The hitherto mentioned blood flow measurements were made on the fingers, where the quantitative variations must be expected to be especially pronounced because of arterio-venous anastomoses. In order to investigate if also other skin localities are involved in the vasoconstriction at the beginning of work, a series of experiments was made, in which skin temperatures were measured on the hands, feet and trunk.

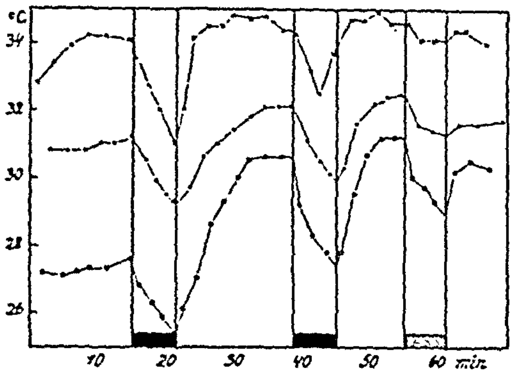

Fig. 4. Subject E. H. C. 1 080 mkg/min.

Skin temperatures
× Finger
● Foot arch
○ Toe

Actual work
No-load experiment

Fig. 4 shows results from simultaneous skin temperature measurements on the finger, arch of the foot and toe, at rest, during work of 1 080 mkg/min. and also in a no-load experiment, that is an experiment in which the subject rides with normal speed but without current on the braking magnets. During the two work periods of 1 080 mkg/min. all three temperature curves show a considerable decrease which is immediately replaced by an increase at the cessation of work. On the other hand during the no-load experiment the finger temperature remains practically unchanged whilst it falls perceptibly on the foot arch and toe. The fall is considerably less than during the two work periods and must be attributed to the increased heat loss by convection caused by the bicycle movements. It is evident from this and corresponding experiments that the feet are also included in the vasoconstriction

at the beginning of work. Similar experiments have shown that also the hands are involved.

The large thermal lag of the trunk makes it very difficult to demonstrate vasoconstriction of short duration as one might possibly expect at transition from rest to work. It is namely known from the experiments with blood flow measurements on the fingers, that the initial vasoconstriction is replaced after a few minutes by a vasodilatation serving the physical heat regulation. Furthermore the secretion of sweat begins after some minutes and hereby brings about a fall in the skin temperature which is independent of changes in the blood flow. In the present experiments with heavy

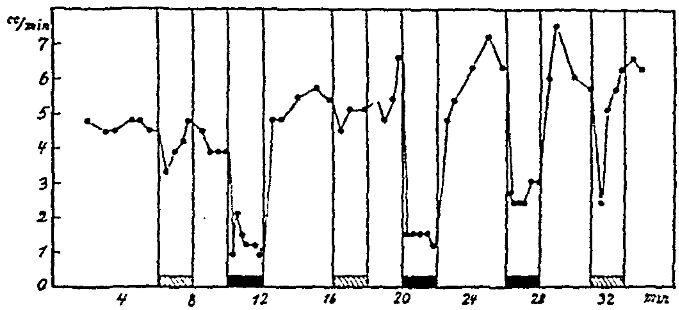

Fig. 5. Subject E. H. C. 1 260 mkg/min. 22° C.
Actual work
No-load experiment

work it is demonstrated that a slight decrease of the skin temperature of the chest and abdomen occurs at the beginning of the work several minutes before the onset of sweat secretion.

Our experiments show therefore that a general reduction of the skin's blood flow is usually to be observed at the transition from rest to work. The question is now whether this decrease should be interpreted as a regulatory reaction as suggested in the introduction or if it can be psychically conditioned and associated with the vasomotor changes that for example appear after unexpected and sudden sound and light stimuli.

In order to investigate this problem we made various control experiments. For this purpose a switch was arranged to the magnetising current in Krogh's cycle ergometer by means of which the experimentor could make and brake the current without the knowledge of the subject.

Fig. 5 shows a series of brief spells of work some with and some without load. The subject was in all cases expecting the same work

intensity, 1 260 mkg/min., but in the experiments without load the current to the braking magnet was not put on. The fig. shows a very marked reduction in the finger's blood flow during real work, but without load the reduction is slight only.

During *light* work, which the subject can easily perform, and even in no-load experiments when the subject is aware that the ergometer is unloaded, we have usually found a slight initial reduction in the blood flow. With repeated starts this decrease often diminishes or completely disappears. The responsible vasoconstriction must be considered as purely psychic and without any regulatory importance. It must be regarded as a somewhat accidental accompanying phenomenon occasioned by the interruption of the resting state that occurs with beginning of the work.

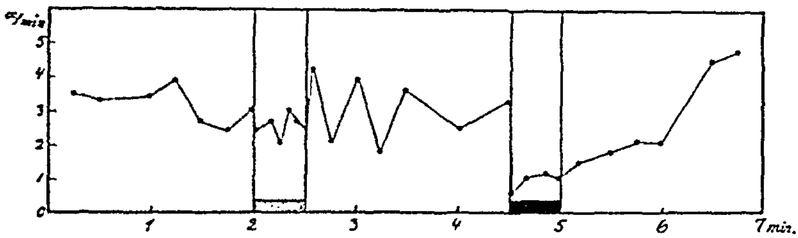

Fig. 6. Subject E. H. C. 1 440 mkg/min.
Blood flow during first seconds of work.
Actual work
No-load experiment

At the start of *heavy* muscular work, however, an instantaneous and vigorous reduction in the finger's bloodflow always occurs and lasts several minutes. Furthermore, with the aid of skin temperature measurements corresponding variations are demonstrated in the hands and feet, and also on large areas of the skin of the trunk although to a lesser degree. From these experimental results we conclude that the vasoconstriction in the skin at the beginning of *heavy* work is of regulatory significance for the adjustment of the circulation at the transition from rest to work.

Fig. 6 gives results from a more detailed investigation of the changes in the blood flow during the first seconds of work. About 1 second after the beginning of work, pressure is applied to the finger cuff so that the first determination during work gives the blood flow in the next second. Thereafter a determination is made every 10 seconds. As is evident from the figure the blood flow is practically unchanged during the no-load experiment, whilst with

work of 1 440 mkg/min. there is a marked reduction and the minimum value is reached already after 1 or 2 seconds. During the subsequent restitution period the blood flow only slowly returned to resting values.

As the reduction in the blood flow is practically instantaneous it can hardly be attributed to humoral influences, as for example secretion of adrenalin, or be evoked reflexly from the presso-sensible zones. One can therefore assume that the vasoconstriction is purely nervous and caused either by cortical impulses or reflexly by impulses from the working muscles.

In numerous experiments it is shown that with no-load one obtains precisely the same slight vascular reaction whether the subject is aware that the ergometer is unloaded or if he is prepared for a work of very high intensity and believes that he starts with this. It is further shown that during riding on the unloaded ergometer, when the current is suddenly connected to the braking magnets without the subject's knowledge, one obtains the same vascular reaction as when the subject changes from rest to heavy work and is prepared for it. It must therefore be concluded, that the expectation of work plays no part in evoking the vasoconstriction. (Compare KROGH and LINDHARD, 1912).

In a series of experiments we have simultaneously with the flow measurements made volume registrations on the neighbouring finger in order to obtain possibly some information regarding the function of the skin as a blood depot. A similar plethysmograph was used as for flow measurements but on account of the longer duration of these experiments water transmission was employed instead of air transmission. The volume changes were read on a nearly horizontal tube of 1.6 mm diameter divided in mm.

Fig. 7 reproduces results from such an experiment with work of 720 mkg/min. The flow curve shows the usual sharp fall at the beginning of work. After about 6 minutes of activity the blood flow increases simultaneously with the subject's heat sensations and the beginning of sweat secretion. During the entire remainder of the period of work the blood flow remains at a considerably higher level than in the preceding resting period.

In contradistinction to the flow curve the volume curve indicates a very gradual decrease and the lowest values are attained 4—5 mins. after beginning of the work. After work has progressed for 6—7 minutes viz. simultaneously to the subject's sensations of warmth and the increase of the flow curve, the volume of the finger

also rises and attains a value that is considerably higher than the preceding resting value. The total finger volume studied was both for the flow measurements and the volume measurements about 12 cc, and the decrease at the beginning of work corresponded to about 0.2 cc below and the volume increase at the end of work to about 0.3 cc above the resting level. The other volume measurements we have carried out have given similar results.

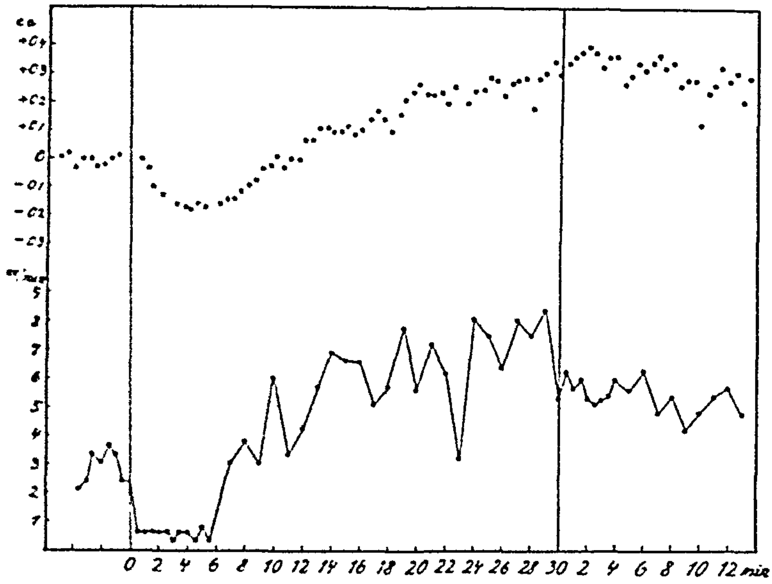

Fig. 7. Subject E. H. C. 720 mkg/min. 23.5° C.
● Blood flow in index finger
○ Volume measurements on third finger

On the basis of these experimental results we conclude that the skin has no significance as an active blood depot at transition from rest to work. If this was the case one would expect a rapid reduction in the volume brought about by venous contractions and corresponding to those changes one assumes to take place in the splanchnic area at the begining of work. Furthermore, such an interpretation would not be in conformity with the considerable increase occurring in the finger volume later in the working period.

These volume changes are possible secondary and contingent upon changes in the blood flow. The reduced blood flow at the beginning of the work may result in a gradual evacuation of the veins and the large blood flow later in the working period in an increased volume. After the cessation of work the finger volume

remains at the high level for a considerable time although the blood flow is decreasing. This indicates that a part of the volume increase during work is due to a filtrate formation that is only slowly reabsorbed. The increased filtrate formation during work may be assumed to be brought about as a result of an increased capillary pressure due to arterial dilatation.

Summary.

At the beginning of heavy work an instantaneous and considerable reduction of the skin's circulation always occurs which subsequently remains low for several minutes. With light work the decrease in the flow is less pronounced and is usually absent after repeated starts. From our experimental results we conclude that the vasoconstriction in the skin is of regulatory significance for the adjustment of the circulation at the transition from rest to heavy work.

As the decrease in the blood flow occurs practically instantaneously at the start of work it is assumed that it is of nervous origin — caused either by cortical impulses or by reflex influences from the working muscles. It is shown that the expectation of work plays no part in evoking the vasoconstriction.

On the basis of experiments with registration of the finger's volume, we believe that the skin has no significance as an active blood depot at transition from rest to work.

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From the Zoophysiological Laboratory, University of Copenhagen.

Measurements of the Blood Flow in the Skin at Rest and during Work at Varied External Temperature.

By

E. HOHWÜ CHRISTENSEN and MARIUS NIELSEN.

(Received 27 April 1942.)

In the present investigation measurements of the blood flow in the skin at rest and during work at varied external temperatures have been carried out by means of BURTON's plethysmographic method in the way described by CHRISTENSEN and NIELSEN (1942).

The measurements were made on the fingers of the subjects, and it must be assumed that the changes in the blood flow of the finger although quantitatively far greater give an indication of the general changes in the blood flow of the skin. In some of the experiments the relation between the subject's sensations of warmth and the changes in the blood flow was studied.

Fig. 1 and fig. 2 show measurements carried out during work of 360, 720 and 1 080 mkg/min at an external temperature of about 15° C and 21° C respectively. The work is performed on the modified KROGH bicycle ergometer, where the subject is placed in a comfortable arm-chair and where both hands can be utilized for measuring. Both subjects show at 15°, a very slight bloodflow when at rest, and even work of 360 mkg/min. is unable to increase it. With work of 720 mkg/min. and still more with work of 1 080 mkg/min. there exists however a considerable increased flow at the end of the period of exertion and the beginning of the restitution period. With a higher external temperature of 21° the flow is on an average far greater both at rest and during work. Here also, the intensity of the work determines the increase that occurs.

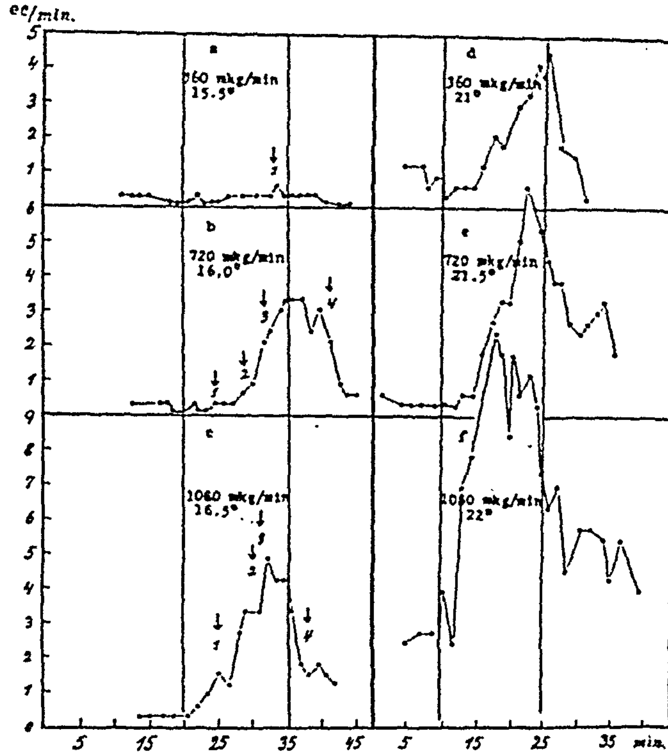

Fig. 1. Subject M. N.

Blood flow measurements in index finger during work at varied external temperature. The times when the subject experienced temperature sensations are marked by numbered arrows.

a 1) subject experiences neutral heat
b 1) neutral heat
2) sensations of heat in the face
3) perspiration on the forehead
4) subject feels cold
c 1) subject feels warm
2) perspiration begins
3) vigorous perspiration
4) subject feels cold

In experiments made at 15°, we have recorded the temperature sensations observed by the subjects to find out whether there is a relation between the blood flow measured in the finger and the sensations of cold or warmth. Our subjects who were very lightly clothed and especially E. H. C. who was only dressed in shorts and a thin shirt, had, in the resting period before the work at this external temperature, definite sensations of cold. With the light work of 360 mkg/min., E. H. C. felt cold during the entire working period and subsequent restitution. This work which is equivalent to an extra calorie production of about 3.5 Cal./Min., or 50 Calories

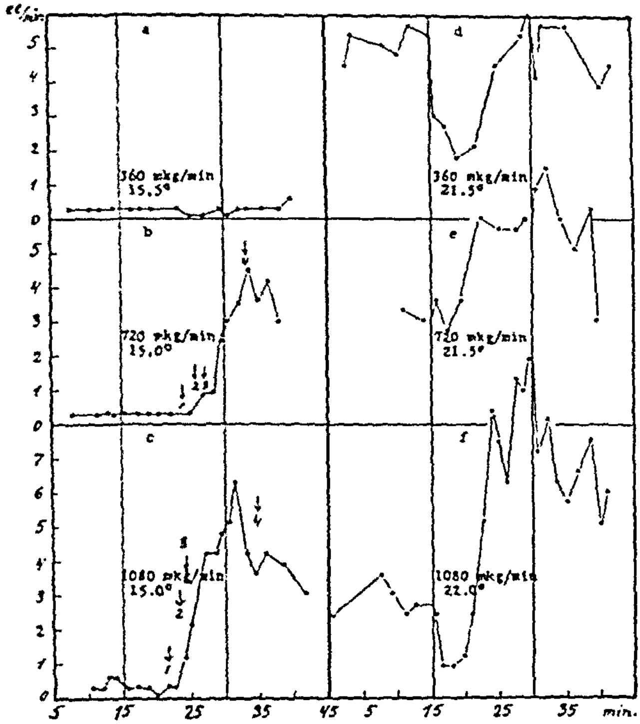

Fig. 2. Subject E. H. C.

Blood flow measurements in index finger during work at varied external temperatures.

a 1) subject feels cold during the entire working period.
b 1) subject feels cold
2) subject begins to feel warm
3) perspiration begins
4) subject begins to feel cold
c 1) subject begins to feel warm
2) perspiration begins
3) vigorous perspiration
4) subject begins to feel cold

in the course of the 15 working minutes was insufficient to remove the subject's sensations of cold and the blood flow remained at the same low level during the whole experiment. In the case of the other subject the sensations of cold disappeared during the last working minutes, but the blood flow remained constantly low during the entire experiment.

As seen from the figures sensations of warmth appear practically simultaneously with the first faint increase of the flow at the two higher rates of work (720 and 1 080 mkg/min.). In the restitution period sensations of cold are observed accompanied by a reduction in the blood flow even when this is still rapid.

It is clearly evident from these results that temperature sensations have no relation to the absolute values of the blood flow but to the latter's increase or decrease.

On the basis of these experiments the cause for the temperature sensations cannot be ascertained, but work experiments with more extensive measurements are considered well suited for the solution of this problem as in a short time one can create pronounced changes in the subject's temperature sensations and simultaneously measure a succession of factors that are conceivably of importance. As such factors the following can be enumerated viz. — absolute values of and increase or decrease in rectal temperature, skin temperature or the blood flow of the skin. Furthermore, one must assume that the velocity with which the changes occur can be of great importance. All these factors can be varied at will in short periods of time by means of work experiments.

Summary.

A series of determinations of the blood flow of the skin of the finger was undertaken at rest and during work at varied external temperature. The results show that even comparatively slight variations in the external temperature are accompanied by very considerable differences in the finger's blood flow. No relation was found between the subjective temperature sensations and the absolute values of the blood flow, but an incipient increase in the flow is attended by sensations of warmth and conversely, an incipient decrease is accompanied by sensations of cold. A programme for a more thorough study of the cause of the sensations of warmth has been outlined.

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Blutdrucksteigerung durch hypoxische Erregung der Chemorezeptoren beim Hund.

Von

H. BJURSTEDT und U. S. v. EULER.

(Eingegangen am 28. April 1942.)

Bekanntlich verursacht Erregung der Chemorezeptoren des Sinusgebietes nicht nur eine starke Anfachung der Atmung, sondern beeinflusst auch den Kreislauf (HEYMANS, BOUCKAERT, EULER und DAUTREBANDE 1932, WINDER, BERNTHAL und WEEKS, 1938). Erhöhung der CO_2-spannung, Säuren und synaptotrope Stoffe bewirken somit reflektorisch eine Vasokonstriktion, Herzbeschleunigung und Blutdrucksteigerung.

In einem Versuch über die Wirkung pressorischer und chemischer Sinusreize auf die Atmung des Hundes haben wir einige Beobachtungen über die Wirkung chemischer Glomuserregung auf den Blutdruck gemacht. Dabei waren Sinus und Glomus der einen Seite durch eine Ligatur dicht oberhalb der Bifurkation zirkulatorisch getrennt und die Barorezeptoren inaktiviert. Absperrung des Externagebietes durch Einbindung einer Kanüle, zwecks Perfusion des Glomus, veränderte die Atmung kaum, was darauf hinwies, dass die Blutversorgung des Glomus aus dem peripheren Teil des A. occipitalis genügend war, um stärkere hypoxische Erregung zu verhindern. (cf. WINDER u. Mitarb.). Nach Einschalten der Perfusion trat sofort eine starke reflektorische Atmungs- und Blutdrucksteigerung ein durch Erregung der Chemorezeptoren (fig. 1 A).

Die Analyse des Perfusionsblutes nach van Slyke (heparini-

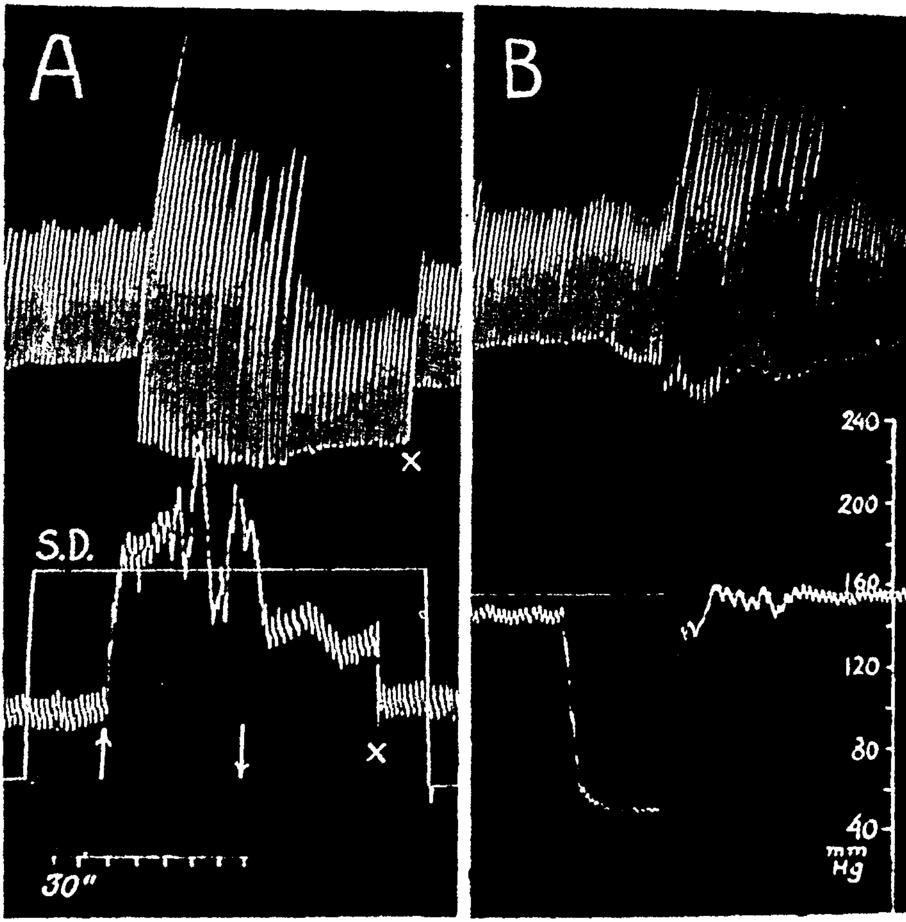

Fig. 1. Hunde, 13 kg, Chloralose. Druck im r. Sinus kontrolliert (S. D.), Barorezeptoren inaktiviert, linkes Sinusgebiet denerviert. Beide Vago-Depressoren durchschnitten A. Rechter Glomus zwischen ↑ und ↓ mit 90 % O_2-gesättigtem Blut (11.5 Vol.% O_2) isoliert perfundiert. B. Allgemeine Blutdrucksenkung durch Auslauf von Blut zum Kompensator.

siertes Schweinevollblut, 6 Stunden früher schlachtfrisch genommen) ergab die folgenden Werte:

O_2-Gehalt	O_2-Kapazität	Sättigungsgrad	CO_2-Gehalt
11.5 Vol.%	12.8 Vol.%	90 %	33.7 Vol.%

Es erscheint zunächst überraschend, dass dieses Blut eine so starke Erregung herbeizuführen imstande war. Bei einem Sättigungsgrad von 90 % ist mit einem wenig herabgesetzten Sauerstoffdruck zu rechnen und trotzdem war die hypoxische Erregung stark. Nach der Ansicht einiger Autoren (Asmussen und Chiodi, 1941) ist die Sauerstoffspannung des Blutes für den Erregungszustand im Glomus ausschlaggebend und nicht der Sauerstoff-

inhalt. Wir möchten gegen diese Ansicht hervorheben, dass die O_2-Spannung nur bei einem genügend hohem O_2-Gehalt den O_2-Verbrauch der erregbaren Strukturen aufrechtzuhalten vermag. Wenn z. B. die Durchströmungsverhältnisse ungünstig sind oder die Sauerstoffkapazität niedrig ist, besteht trotz einer hohen O_2-Spannung des umgebenden Blutes die Gefahr einer Hypoxie. In dem angeführten Versuch ist die niedrige O_2-Kapazität des Perfusionsblutes als wichtigste Ursache der lokalen Hypoxieerregung anzusehen, da man mit etwa dem doppelten Wert beim Hund rechnen darf. Eine CO_2-Erregung ist wegen des niedrigen CO_2-Gehaltes nicht anzunehmen.

Aus der Figur ist ferner ersichtlich, dass sobald der reflektorisch erhöhte Blutdruck einen gewissen Wert überschritten hatte, wobei Glomus wieder mit körpereigenem Blut retrograd versorgt wurde, sowohl Blutdruck als Atmung schnell heruntergingen, um dann wieder zu steigen, wenn der arterielle Druck im peripheren Teil der A. occipitalis niedriger war als der Perfusionsdruck. Nach Abstellung der Perfusion sanken Blutdruck und Atmung bald auf ihr früheres Niveau.

Eine ähnliche reflektorische Wirkung konnte bei Erniedrigung des allgemeinen Blutdruckes durch Auslauf von Blut in eine Kompensationsvorrichtung (bei abgestellter Glomusperfusion) beobachtet werden (Fig. 1 B). Nach Inaktivierung der Chemorezeptoren traten diese Wirkungen nicht mehr ein. Die Bedeutung der Sauerstoffsättigung des eigenen Blutes ging daraus hervor, dass bei Sauerstoffatmung die Atmungssteigerung nach allgemeiner Blutdrucksenkung etwa 1 Minute später eintrat.

Zusammenfassung.

Hypoxische Erregung des isoliert perfundierten Glomusgebietes beim Hund mit ausgeschalteten Barorezeptoren führte sowohl eine starke Atmungsteigerung als auch eine sehr ausgesprochene Blutdrucksteigerung herbei.

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Assay of Digitalis Preparations by the Guinea-Pig Method.

By

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(Received 8 May 1942.)

The guinea-pig method for standardization of cardiac glucosides, founded on the cat method of HATCHER and BRODY (1910), was worked out by KNAFFL-LENZ (1926). — In order to obtain constant values, KNAFFL-LENZ (1926, 1928) gave certain directions: The animals must have a weight of 500—800 g, and after 6—8 hours starving must be anaesthetized with ether, which is administered by means of artificial respiration. The concentration of the solution injected should correspond to 1.0—2.5 % fol. dig. and be administered at the rate of 0.5—0.6 ml per min, this being reduced to 0.1—0.2 ml per min, when the action of the heart begins to be irregular. The end point: cardiac failure in systole with no mechanical excitability, then appears after 15—20 min. — The concentration of the unknown sample is to be chosen so that the amount required for the standstill shall not exceed 1.0—1.5 ml per 100 g (animal). Under these circumstances a *guinea-pig unit* is defined as the amount of drug required per 100 g for the cardiac failure.

Anaesthesia with ether caused rather large deviations in the lethal doses, and after examination of several anaesthetics KNAFFL-LENZ (1931, GADDUM 1932) found, that in a dose of 1 g per kg in a 25 % solution urethane was most convenient. Subsequently, however, 1.5—2.0 g urethane per kg has been used (BURN 1937). — MARRI (1939) and MARRI and CIAPPI (1939) compared the potency of dig. purp. and dig. lan. on non-anaesthetized animals and on

animals anaesthetized with urethane, but were not able to prove any statistically significant difference.

Concerning the *weight* KNAFFL-LENZ (1926) emphasized that animals of less than 500 g seemed able to stand proportionately more of the drug than those with a normal weight, others of over 800 g proportionately less.

The *concentration* of the solution injected has been assigned different significance for different drugs. When using fol. dig., the quantity required was larger with a 0.5 % solution than with 1.0—2.5 % solution (KNAFFL-LENZ 1926), in the case of strophantin and Verodigen, however, the amount required increased with the concentration (GADDUM 1932, STRAUB 1941). OTTERSTRÖM (1934) and LÉVY and OTTERSTRÖM (1934), on the other hand, could not prove any changes in the lethal doses with dig. purp., when the concentration varied.

The *rate* of injection has not been considered of any great importance for dig. purp., while when using Digifolin ITZINGER, KNAFFL-LENZ and REISCH (cit. KNAFFL-LENZ [1931]) found the lethal dose to be inversely proportionate to the amount of drug injected per min and 100 g (cp p. 190).

OTTERSTRÖM (1934) and LÉVY and OTTERSTRÖM (1934) found when investigating the *duration* of the injection that the standard error was slight, when the rate of injection was regulated so that the experiment lasted for 15 min, and increased with rising duration (30—80 min.). They could not prove any change in the lethal dose with different times of injection, neither could BRUN (1939), who varied the length of the experiment from 12 to 57 min; STRAUB (1941), on the other hand, found the time of infusion for Verodigen and k-strophantin to vary with the concentration of the solution used.

Compared with the frog assay, the guinea-pig like the cat method has the advantage of requiring fewer animals and of being usable the whole year round, and finally of having less variability.

The accuracy of the guinea-pig method has varied somewhat in the hands of different workers: the coefficient of variation has been 10—15 % (TREVAN, BOOCK, BURN and GADDUM 1928, KNAFFL-LENZ 1928, OTTERSTRÖM 1934, GRAM 1938, MARRI and CIAPPI 1939, BRUN 1939, STRAUB, KANDA and ZINNITZ 1940), *i. e.* corresponding to the accuracy of the cat method (LIND VAN WIJNGAARDEN 1926, BLISS and HANSON 1939).

No extensive investigations, however, have been made on the *variations* of the guinea-pig unit. STRAUB, KANDA and ZINNITZ state: "Saisoneinflüsse haben wir an den Meerschweinchen weder während der kalten noch der warmen Jahreszeit bemerkt. Die Tiere waren im grossen und ganzen gleichmässig empfindlich." At the same time they say that when an animal showed a divergent value, it was due to "einen Lungenbefund". REISER (1940) claims that pregnant animals give unsatisfactory values and recommends males for standardization while STRAUB (1941) finds no variation of the guinea-pig unit.

Experience shows that the lethal dose, when determined by the guinea-pig method, can vary from time to time. No unanimous conception has been arrived at as to what conditions cause the variations. This work is intended to analyse a few of the causes of these variations and to determine their magnitude and significance for the accuracy of the guinea-pig method for the assay of samples of unknown potency.

Experimental.

Methods.

Guinea-pigs, weighing 229—685 g, were used for the experiments, generally 6—8 animals in each group. Coming from the dealer, the animals were kept in the laboratory for a week in order to obtain a constant weight (GADDUM 1932), and the experiments were usually made without the animals having to be starved.

I. Anaesthesia with 1.75 g urethane per kg in a 25 % solution. After 2 hours preparation *ad modum* KNAFFL-LENZ (1926). Artificial respiration from the first. The chest was opened, when the action of the heart was becoming irregular. End point: cardiac failure in systole with no mechanical excitability.

II. Anaesthesia with 2.0 g urethane pr kg in a 20 % solution, preparation after 1 hour, spontaneous respiration until respiration ceased, then artificial respiration and opening of the chest, end point as above.

The injection was made by means of a micro-burette at a constant rate of infusion throughout the experiment, and varied from test to test: 0.10—2.0 ml per min, according to table 1.

The following preparations served as test samples:

1. Digisolvin Leo: potency 2.7 guinea-pig units per ml, corresponding to 44.28 mg fol. dig. int. stand. (1936). Concentration used: 11.5—66.5 %, corresponding to 0.51—2.22 % fol. dig. int.

2. Fol. dig. nat. stand.: potency 132.4 % of the international standard (1936), concentration 1.45—2.0 %, corresponding to 1.92—2.65 % fol. dig. int.

I. Examination of the Knaffl-Lenz' Method.

The factors firstly investigated were the effect of the varying rate and time of the injection, the weight of the animals and finally different concentrations of the solution infused (part I). Digisolvin served as a test sample in this series, and according to method I the experiments were carried out at the State Pharmaceutical Laboratory, Oct. 1940—Febr. 1941. In a second part (II) the variations of the guinea-pig unit at repeated determinations are examined.

1. Varying Rate and Duration of the Injection.

A typical series of experiments on 7 groups of animals, illustrating the influence of the varying rate of injection on the size of the lethal dose is given in table 1. The concentration of the solution injected corresponded to 1.26 % fol. dig. int., the rate of injection varied from 0.15 to 1.0 ml solution per min, and the duration of the infusion varied from 9 to 48 min; the mean weight of the animals was 449 g.

From table 1 will be seen that the lethal dose *varied* with the variations of the injection rate, and *increased* with increasing rate of injection.

The same tendency: augmenting lethal dose with increasing injection rate, also was found in experiments on 4 groups, the animals weighing only 229—302 g with a mean weight of 257 g; the injection rate varied from 0.10 to 0.77 ml per min, the concentration being as before 1.26 %.

The experiments are illustrated in fig. 1 A, where log lethal doses (group mean values) are plotted against injection rate.

The relation between the lethal dose and the rate of injection seems to be rectilinear; the curves of the two weight series 449 g and 257 g respectively showed a certain tendency to coincide, with the exception of the highest values.

A statistical treatment of the series showed, however, that the two curves differed more from each other than was due to chance (P = 0.01[1]). Another principle of classification was therefore tried, in which the varying weight of the animals was taken into

[1] P = the chance of probability, calculated by means of the variance ratio according to SNEDECOR (1938) and BONNIER and TEDIN (1940).

consideration: the lethal dose was related to the amount of drug injected per min and 100 g. In fig. 1 B the log lethal doses (group mean values) are plotted against log mg drug injected per min and 100 g.

Fig. 1 B shows, that the log lethal dose increased with increasing amount of drug injected per min and 100 g, and rectilinear as it would seem. The two curves showed a pronounced tendency to coincide. Thus no difference could be proved statistically (P =

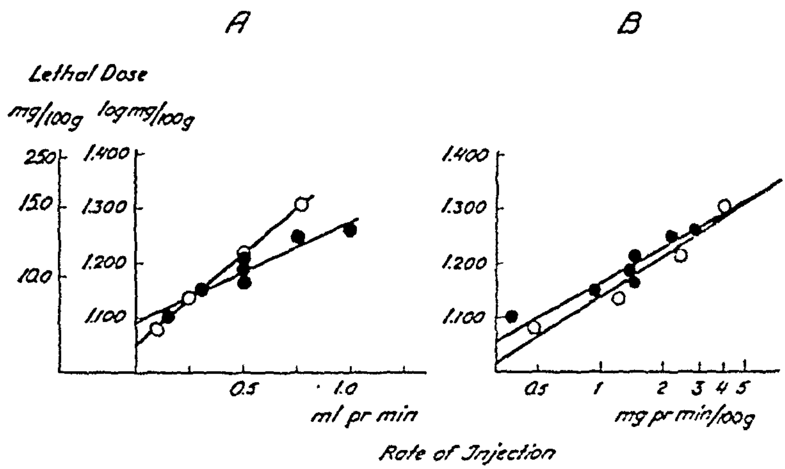

Fig. 1.

A. Log lethal dose (mg fol. dig. int. pr 100 g) plotted against rate of injection in ml per min of a 1.26 % solution.

B. Log lethal dose (mg pr 100 g) plotted against rate of injection in log mg fol. dig. int. pr min and 100 g.

●——● mean weight 449 g
○——○ » » 257 g

0.2). With this classification the weight of the test animals could vary within broad limits (229—685 g) without the size of the lethal dose being affected.

The *duration* of the injection varies inversely in proportion to the rate, and in fig. 2 A the relation is illustrated between the log lethal dose and the time of infusion. From the graph it will be seen that the lethal dose (or log lethal dose) *changed* with variations in the duration of the experiment: it decreased with increasing time of injection, after a logarithmic curve, as it seems, and the curves for the two weight series differed from each other considerably during the whole of their course.

If the lethal dose (or log lethal dose) was related to the time of injection per 100 g, thus taking the varying weight of the animals

into consideration, the two curves now as well were seen to decrease with increasing time of injection, according to a logarithmic curve. But now they showed a certain divergence only in the first part of their course, up to 2.0—2.5 min per 100 g, after which they completely coincided. The cause of this difference with short injection times will depend on the technique adopted:

At the end of the injection, when the action of the heart begins to slow down, the rate of injection will be diminished to 0.1—

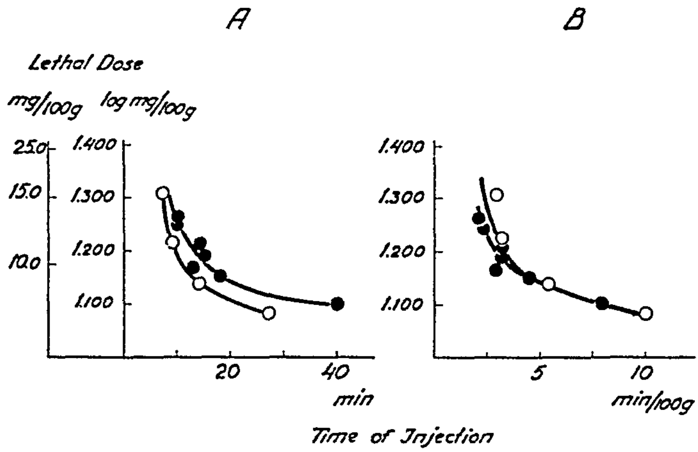

Fig. 2.

A. Log lethal dose (mg fol. dig int. pr 100 g) plotted against time of injection in min.
B. Log lethal dose (mg fol. dig. int. pr 100 g) plotted against time of injection in min/100 g.

●——● mean weight 449 g
○——○ » » . 257 g

0.2 ml per min, irrespective of what it has previously been. The time elapsing from the moment the rate is diminished till the heart stopps scarcely varies with the size of the animal, and it is therefore percentually of greater importance with a small animal, where the total time is shorter. The time of injection per 100 g will thus be relatively too long at short injection times and with small animals, giving the curve a steeper slope up to 2—2.5 min per 100 g.

2. The Weight of the Animals.

The importance of the variations of the weight for the size of the lethal dose now becomes clear.

The *rate* of injection being *constant*, the lethal dose becomes larger, the smaller the animal, because the amount of drug injected

per min and 100 g increases. This confirms the principal findings of KNAFFL-LENZ (1926). Between 350 and 500 g the difference in weight is of little practical importance with a solution, corresponding to 1.26 fol. dig. int., injected with a rate of 0.25—0.30 ml per min, but it is clearly apparent at an increased rate of injection or with a higher concentration. — This is illustrated in fig. 1 A, the curve of mean weight 257 g lying above that of 449 g at a higher injection rate than 0.30 ml per min.

If the *total time* of injection is kept constant, the lethal dose decreases with the size of the animal, the amount of drug injected per min and 100 g diminishing with decreasing weight (fig. 2 A).

The rate as well as the time being kept constant *per 100 g* (fig. 1 B and 2 B resp.) the weight curves will be seen to coincide, and this means that the lethal dose (or log lethal dose) under these conditions is not affected by the weight of the animal.

3. The Concentration of the Solution Injected.

In order to examine the importance of the concentration, the experiments were repeated with 5 different concentrations, corresponding to 0.51—2.22 % fol. dig. int., and varying rates of injection within each concentration: 0.10—2.0 ml per min, on a total of 24 groups of animals. The mean values of the lethal doses (in ml test) with other data are to be found in table 1.

The results were: The rate of injection being kept constant, the lethal dose increased with increasing concentration, which is due to the fact that the amount of drug injected per min and 100 g increases; this confirms GADDUM's and STRAUB's statements on strophantin and Verodigen (cp. p. 179). — The concentration being constant, as before an increase of the lethal dose was to be found with increasing rate of injection.

The standard deviation within the groups varied from 7.6 % to 22.8 %, on an average 11.6 %, when expressed in per cent of the mean, *i. e.* = the coefficient of variation. The standard error of the mean varied from 2.9 % to 9.1 % with an average of 4.5 %, 6—7 animals being used in each group. The coefficient of variation was thus of the same magnitude as that found by other workers (cp p. 179) and somewhat lower than that found for the cat method (LIND van WIJNGAARDEN 1926, BLISS and HANSON 1939).

The standard deviation was somewhat greater with lower concentrations (0.51—0.55 %), on an average 14.4 %, than with

Table 1.

Lethal Doses of Digisolvin after Variations in Concentration, Rate of Injection and Weight.

Concentration fol. dig. int. %	Test Animals		Rate of Injection		Time of Injection		Lethal Dose	
	Number	Weight in g Range and Means	ml/min	mg fol. dig. per min/100 g	min	min/100 g	ml test/100 g	Coeff. of Variation %
0.51	8	421—485 (456)	1.25	1.39	13	2.9	0.323 ± 0.026	22.8
›	3	536—578 (552)	2.0	1.88	13	2.4	0.360 ± 0.012	9.4
›	8	446—505 (480,	›	2.11	11	2.3	0.362 ± 0.011	11.0
0.55	5	389—522 (450)	0.25	0.33	40	9.6	0.281 + 0.008	8.6
›	5	389—440 (417)	0.70	0.88	22	4.9	0.318 ± 0.029	20.4
1.26	8	255—302 (266)	0.10	0.47	27	10.0	0.274 ± 0.012	12.4
›	9	229—299 (261)	0.25	1.22	14	5.4	0.310 ± 0.013	12.6
›	9	238—288 (263)	0.50	2.44	9	3.2	0.374 ± 0.015	12.0
›	8	225—280 (248)	0.77	3.97	7	2.9	0.462 ± 0.019	11.6
›	5	469—579 (509)	0.15	0.38	40	7.9	0.288 ± 0.011	7.6
›	5	398—434 (410)	0.30	0.93	18	4.5	0.321 ± 0.013	9.1
›	8	429—520 (461)	0.50	1.38	15	3.2	0.350 ± 0.012	9.3
›	8	427—464 (444)	›	1.42	13	2.9	0.334 ± 0.011	9.3
›	8	391—475 (439)	›	1.45	14	3.2	0.367 ± 0.014	10.8
›	8	395—497 (433)	0.75	2.21	10	2.3	0.401 ± 0.019	13.4
›	6	430—464 (440)	1.0	2.84	10	2.1	0.416 ± 0.022	12.9
2.02	8	400—472 (427)	0.20	0.95	17	4.0	0.323 ± 0.011	9.7
›	10	396—500 (466)	0.40	1.94	12	2.6	0.407 ± 0.018	16.0
2.22	4	451—468 (460)	0.10	0.46	26	5.7	0.262 ± 0.010	7.6
›	5	380—544 (455)	›	0.50	23	5.1	0.238 ± 0.009	8.4
›	4	670—685 (678)	0.50	1.64	15	2.2	0.384 ± 0.017	8.9
›	4	387—521 (458)	›	2.45	10	2.2	0.374 ± 0.019	10.2
›	5	406—425 (414)	›	2.68	10	2.4	0.375 ± 0.017	10.7
›	3	361—383 (372)	›	2.98	8	2.1	0·392 ± 0.032	14.1

stronger solutions: 11.6 % with a 1.26 % solution and 10.7 % with 2.0—2.22 % solutions. No statistically significant difference in standard deviation could be proved between slow and rapid rate of injection, as OTTERSTRÖM (1934) and LÉVY and OTTERSTRÖM (1934) claim.

In order to compare the effect of the different concentrations

and rates of injection the log lethal dose, expressed in mg fol. dig. int. per 100 g, was related to the amount of drug injected per min and 100 g, expressed in mg fol. dig. int. too, and plotted on a graph (fig. 3 A).

From fig. 3 A it will be seen that all values grouped themselves along a straight line, the regression line (cp p. 195).

The lethal doses (or log lethal doses) showed a considerable increase with increasing amount of drug injected, and the determining factor for the magnitude of the lethal dose seemed to be the amount of drug injected per time unit and *100 g, i. e.* the product of the rate of injection and the concentration.

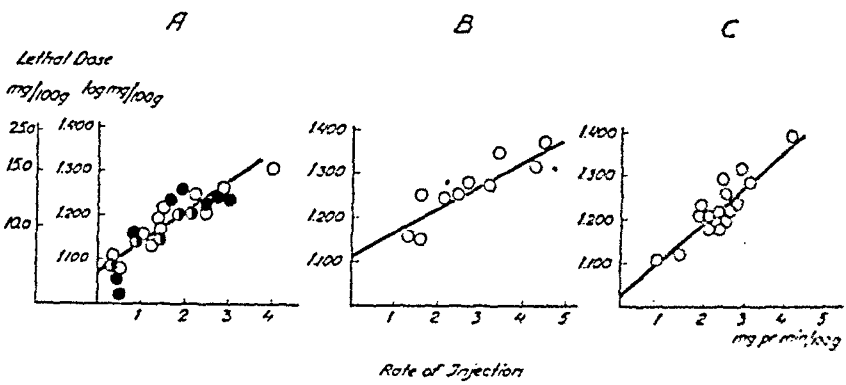

Fig. 3.

A. Digisolvin. B. Fol. dig. nat. stand. (K. I.). C. Fol. dig. nat. stand. (S. P. L.). Log lethal doses, group means in mg fol. dig. int., plotted against rate of injection in mg fol. dig. int. pr min and 100 g. Regr. lines calculated from regr. eq. for each series.

Some examples of the size of the changes in the lethal dose when the rate of the injection, the concentration and the weight is varied, are given below. The values are calculated according to formula (3), and as a standard the lethal dose has been chosen for a 2 % solution of fol. dig. int., injected at the rate of 0.5 ml per min on an animal of 450 g, corresponding to an injection rate of 2.22 mg fol. dig. int. per min and 100 g, = 16.65 mg fol. dig. int. per 100 g.

When the weight of the animals is kept constant, an increase of the *rate* of injection from 0.5 to 1.0 ml per min increases the lethal dose to 23.50 mg per 100 g, corresponding to an increase of 41.1 %; a decrease in the *weight* from 450 to 250 g increases the lethal dose by 32.4 %, and finally a decrease in the *concentration* of the solution used from 2.0 to 1.0 % reduces the lethal dose by 15.8 %.

An average time of injection of 12 min corresponds to the standard lethal dose of 16.65 mg per 100 g used above, and variations in this time have a similar effect. The lethal dose increases by 56 %, when the time of the experiment is reduced to 6 minutes, whereas an increase of the time to 35 min only increases the lethal dose by 24.8 %.

The importance of these factors for determining the potency of an unknown sample is illustrated in table 2. The potency is expressed in guinea-pig units, and the factors varied within the limits of the original KNAFFL-LENZ' method, the lethal doses being calculated according to formula (3).

Table 2.

Potency in Guinea-Pig Units of Fol. Dig. Int., When Tested According to Knaffl-Lenz.

Concentration of solution %	Weight of animals g	Rate of Injection		Lethal Dose mg fol. dig./100 g	Guinea-Pig Units pr g fol. dig.
		ml/min	mg fol. dig pr min/100 g		
1.00	800	0.50	0.63	13.02	76.8
1.75	650	0.55	1.48	14.85	67.3
2.50	500	0.60	3.00	18.79	53.2

II. The Variations of the Guinea-Pig Unit at Repeated Determinations.

A. Digisolvin.

As the lethal dose has been defined, it is at the same time a determination of the size of the guinea-pig unit. The changes of the lethal dose at repeated intervals consequently give the variations in the guinea-pig unit, which are to be attributed partly to experimental errors, partly to its own possible variation.

The experimental error was on an average 12.2 %, and the total error 9.2 % (table 3).

By adjusting the lethal dose to the identical amount of drug injected per min and 100 g, according to the regression formula (p. 195) the total error was reduced on an average by 33 %. This reduction consisted mainly of a decrease of the variation from time to time and only to a small extent of differences in the experimental errors before and after adjustment (table 3).

The experiments in this series thus indicated that a statistically significant variation from time to time remained, even when using 6—8 animals for each determination and making adjustments for the variations in the rates of injection.

In order to see whether the circumstances found hold good for fol. dig. as well and not only for a special preparation, containing above all the water-soluble fraction of the digitalis glucosides, the investigation has been repeated with fol. dig. nat. stand., firstly according to method II on animals, some of which come from different sources (B), and secondly under the same conditions as the Digisolvin series (C).

B. Fol. Dig. Nat. Stand. (K. I.).

According to method II the lethal dose was determined at the Pharm. Dep. of Karolinska Instit. from Sept. to Dec. 1940, fol. dig. nat. being used as test on 10 different occasions on altogether 38 animals. The concentration of the solution injected was 1.5 % fol. dig. nat., corresponding to 2.0 % fol. dig. int. (p. 180), the rate of injection varying from 0.35 to 1.0 ml per min.

The lethal dose was found to be 14.40—23.50 mg fol. dig. int. per 100 g, the average being 18.76 mg per 100 g (mean values of 3—5 animals in each group).

As in the Digisolvin series the guinea-pig unit had a variation from time to time that exceeded the experimental error (table 4).

If the log lethal doses were related to the rate of injection per min and 100 g, they varied proportionately with the variations of the amount of drug injected (fig. 3 B).

The *adjusted* mean values (regr. formula [6]) only varied from 16.80 to 20.60 mg fol. dig. int. per 100 g, the total error thus being reduced by 44.7 % as compared with the original values found, and the variation from time to time now being within the limits of error (table 4).

C. Fol. Dig. Nat. Stand. (S. P. L.).

The variations of the guinea-pig unit were followed according to method I from Febr. 1940 to Oct. 1941 at the State Pharmaceutical Laboratory. The determinations were made on 16 different occasions on altogether 85 animals, the rate of injection being constantly 0.5 ml per min (exceptions see below), the concentration 1.45—2.0 % fol. dig. nat., corresponding to 1.92—2.65 % fol. dig. int.

The mean values of the lethal dose, expressed in mg fol. dig. int., 3—6 animals in each group, varied from 15.42 to 20.64 mg per 100 g, the average being 16.90 mg (12 groups, 4 additional groups not included, see below).

The lethal doses, in this series too, varied from time to time (table 5).

Relating the log lethal doses to the rate of injection showed that they varied with the variations in the injection rate (fig. 3 C).

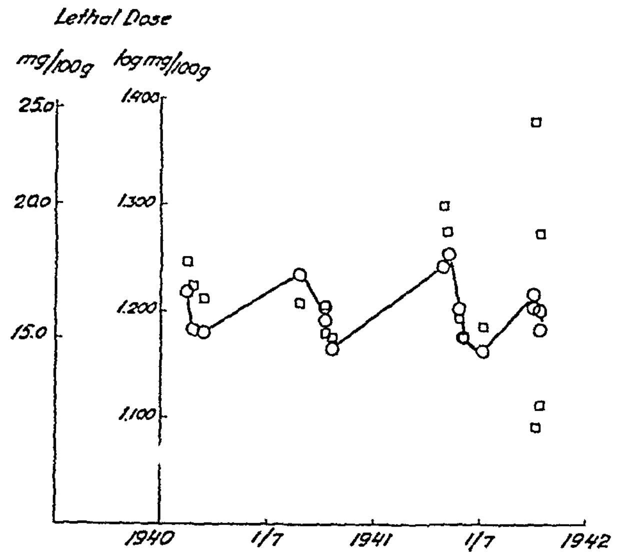

Fig. 4.

Secular variations of log lethal dose. Test: Fol. dig. nat. stand. Log lethal dose, expressed in mg fol. dig. int., plotted against time.

□ mean of actual (or found) log lethal doses
○ » » log lethal doses, adjusted by regr. formula.

A calculation of the regression equation could not be made from the original series observed — 12 groups with 54 animals —, the amount of drug injected per min and 100 g having too small a variation in relation to that of the lethal dose. Consequently 4 another groups of determinations were made on 31 animals with a 1.5 % solution of fol. dig. nat., corresponding to 2 % fol. dig. int., and varying rates of injection: 0.20, 0.35, 0.75 and 1.0 ml per min. The lethal doses were 12.71, 13.31, 19.36 and 24.60 mg fol. dig. int. per 100 g respectively, being mean values of 7—8 animals in each group.

According to the regression equation calculated (p. 198), the total variation decreased by 44.2 % after adjustment, which variation still exceeded, however, the experimental error (table 5).

A diagram of the variations in the mean lethal doses, according to dates of test, is given in fig. 4, from which will clearly be seen the absence of a seasonal trend.

Discussion.

The relation between the lethal dose (or log lethal dose) and the amount of drug injected per min and 100 g indicates that the effect of the drug administered does not set in momentarily but appears after a certain period of latency. The cause will depend on the effect that the cardiac glucosides produce in the body: they are not bound directly and immediately to the cardiac muscle but via an intermediary: a fixation to the serum albumin, from which they will afterwards be given off to the cardiac muscle according to HAARMANN, HAGEMEIER and LENDLE (1940) and HAARMANN, KORFMACHER and LENDLE (1940). Moreover the ability of the cardiac muscle to fix the drug will be limited; on the supply of a larger quantity of drug than can be fixed the surplus must circulate in a free or bound form in the blood and will increase with the rate of injection. This phenomenon is reflected in the increase found of the lethal dose with augmenting quantity of drug injected per time unit.

The period of latency has been observed by BLICKENSDORFER and MCGUIGAN (1940), who at regular intervals injected a test solution of digitalis in dogs; they did not, however, attach any importance to it. EDMUNDS, MOYER and SHAW (1937) with dig. purp. and VOS and DAWSON [quoted from SNEDECOR (1938)] with oubaine have found a change in the lethal dose with variations of the injection rate on cat. On guinea-pigs with Digifolin ITZINGER, KNAFFL-LENZ and REISCH (1931) [cit. KNAFFL-LENZ (1931)] stated that the lethal dose was inversely proportionate to the amount of drug injected per min and 100 g[1], they were not able to show that fact for fol. dig. or any other glucosides, and therefore assumed this proportionality to be specific for that special sample. The experiments in this work, however, indicate that the relation between the lethal dose and the amount of drug injected per min and 100 g is a general phenomenon, and they confirm the graph on Digifolin, given by KNAFFL-LENZ et al. (1931), further

[1] The original work is not to be had, but from a published graph (KNAFFL-LENZ (1931)) it will be clearly seen that the lethal doses and the rate of injection are *directly* proportionate.

the conclusions to be drawn from the *original* values in the thesis of OTTERSTRÖM (1934), which suggest the same thing with fol. dig., digitaline and scillarene A, and to a certain extent with ouabaine, and finally the principal results of STRAUB (1941) with verodigen and k-strophantin.

The calculated *regression equation* (p. 195) will be a characteristic of the effect of the drug on the animals used, and the differences between different drugs and series of animals are reflected by the changes in the *constant* term of the equation, which will be an expression of the varying susceptibility of the animals, and changes with the drug, the stock of animals, and the method adopted.

The regression coefficient, on the other hand, does not seem to vary more than at random within a homogenous stock of animals. In those cases, where it has shown a considerable deviation, this has been due to a heterogenous stock, and the heterogenous groups having been eliminated, its magnitude has not shown any departure from the average beyond the experimental error. From these facts the regression coefficient may be assumed to express the changes in the lethal dose with the *variations* of the injection rate, and characterizes the drug and its mechanism of fixation in the system. It has still to be proved to what extent the size of the coefficient varies when using different drugs.

Finally it may be said that the probability of a *rectilinear* relation between the log lethal dose and the amount of drug injected per min and 100 g seems to be greater than for any other function, at least as far as this work is concerned (the test of deviation of group means from linear regression giving $P = 0.2$).

The *individual* variation, apart from being due to experimental errors, *e. g.* changes in the injection rate *during* the experiment, different end points etc., must also be attributed to differences in the *tolerance* of the individual animal to the drug, the causes of which still are to be investigated (differences in sex, in vitamin balance etc.). Starving the animals before the experiment has proved of no importance (unpublished).

If the rate of injection, the concentration and the weight of the animal were kept constant, the standard deviation for all the series — 50 groups with altogether 277 animals — was on an average 11.2 %, which corresponds to a standard error of the mean of 4.6 %, 6 animals being used for each determination. If all animals were taken from the same source, the standard error was still less: 2—3 % (unpublished). If the

constancy was not kept, the standard deviation increased to 19.4 %, which corresponds to a standard error of 8.0 %, thus making an increase of 73.7 %.

When determined at *repeated* intervals, the lethal dose varied on an average by 18.3 %, 5 animals being used in each group. If the lethal dose (or log lethal dose) was related to the rate of injection, concentration or weight, it varied in proportion to the changes in the rate and the concentration, and inversely in proportion to weight, *i. e.* proportionately to the *quantity* of drug injected per min and 100 g. The adjustment by regression of the lethal doses eliminated about 60 % of the variation. Nevertheless the variations in two of the series, about 7.4 %, exceeded the experimental error. As the technique in each series has been uniform the whole time (the variations in the injection rate eliminated by means of the regression equation) and as all the animals in one group have come from the same source, the changes will be due to fortuitous variations in the susceptibility of the animals, and common to a whole batch.

The mean log lethal doses of the three series, adjusted to the identical rate of injection (2.22 mg fol. dig. int per min and 100 g), were 16.65, 17.26 and 16.37 mg fol. dig. int. per 100 g respectively, showing no statistically significant differences from each other. The slight difference between the two methods adopted thus seems to have been of no importance for the size of the lethal dose nor for the accuracy of the assay, the standard deviations being of the same magnitude (tables 3, 4 and 5).

Conclusions.

The practical consequences concerning the guinea-pig method will be as follows:

If the Knaffl-Lenz directions are followed, the guinea-pig unit of lethal dose can vary by max. 44.3 %, solely by variations in the quantity of drug injected per min and 100 g, the individual variation not being included (table 2). When using 6 animals for each test, the standard error of the mean amounts to 8—10 % on an average. If the variations of the injection rate are eliminated, which is most simply done by keeping the rate of injection, the concentration and the weight constant, or by adjusting the values according to the regression formula, calculated for each

special series, the standard error decreases to 4—5 % on an average, the same number of animals being used the whole time.

As the guinea-pig unit can vary from time to time by max. 20 %, even after adjustment, these variations can be eliminated by determining the size of the guinea-pig unit on a standard preparation with the technique used in the laboratory. — The concentration of the solution, the rate of injection and the weight of the *animals must be kept constant, not only within* each group but also from time to time in order to obtain a minimal standard error and to avoid complicated calculations.

A rate of injection of 2.0—2.5 mg fol. dig. int. per min and 100 g, corresponding to a concentration of the solution of 1.5—2.0 %, when injected at the rate of 0.5 ml per min on an animal of 400—500 g, have proved to be appropriate *standard* conditions. Concentrations of less than 1 % give greater experimental errors as also solutions exceeding 2.5 %. A suitable weight will be 350—500 g, and to simplify matters, if the rate of injection is kept constant per whole animal, the variation in weight within the group should not exceed 50—75 g. The average time of injection will thus be 12—14 min and the average lethal dose 16—18 mg fol. dig. int. per 100 g, varying somewhat with the establishment of the moment of cardiac failure. The consumption will be c. 1 ml per 100 g, and the concentration of the solution will thus correspond to the lethal dose or one guinea-pig unit per ml.

The procedure when standardizing an unknown sample will be: The tolerance of the animals, *i. e.* the size of the guinea-pig unit, is determined with a standard preparation on 6—8 animals. Then the unknown sample should be tested on the same stock of animals as the standard and of the same average weight. The concentration should be chosen as to contain one lethal dose, corresponding to one guinea-pig unit or 0.20—0.25 intern. units, per ml, and should be injected at the identical rate as the standard. If the average times of injection are identical, the values found are directly comparable and the method is suitable for testing samples of different drugs.

Experience shows that slight variations in time and weight are of less consequence than is the constancy of the injection rate. If the time of injection is considerably *shorter* than that of the standard, say by more than 2—3 min, the sample should be diluted, and the whole determination repeated with the new solution; it is the same thing if the time is considerably *longer*, say by more

than 4—5 min, when a stronger solution should be used. — Under these conditions no adjustment for the variations in the amount of drug injected per min and 100 g is necessary.

The *number* of animals required for each test depends on the demands of accuracy established. A determination of the standard on 6—8 animals from a homogenous stock gives a standard error of 3—5 %, which will be suitable if the stated potency of the sample is to be allowed a tolerance of 20—25 %.

As the size of the guinea-pig unit varies with the amount of drug injected per min and 100 g, eventually with the method adopted at the different laboratories in spite of certain regulations being established, and finally from time to time by changes in the susceptibility of the animals, it will be unsuitable to state the potency of a sample in guinea-pig units. By comparing a sample with a national or international standard preparation, the importance of which has already been stressed (VARTAINEN 1932, Quart. Bull. Health Organisation 1935), and by stating its potency in intern. units or g of a standard preparation this uncertainty in the determination is avoided, and makes it possible to every worker in this field to vary his technique to a certain degree as suits him best. Standard preparation and sample must be treated uniformly and be tested simultaneously on the same stock of animals, particularly, if these are coming from different sources or if a considerable length of time elapses between each test. This is absolutely essential when establishing the potency of a new standard preparation, but may be modified within certain limits, when control etc. is concerned.

Statistics.

A. Digisolvin.

The lethal doses as well as the injection rate were expressed in mg fol. dig. int. per 100 g, converted to logarithms and related to the amount of drug injected, expressed as mg fol. dig. int. per min and 100 g, in order to make a comparison possible of the results of this series with other drugs. — The log lethal doses are generally normally distributed and can be transformed to percentage mortality in order to get a mortality curve for calculation of LD 50 (GADDUM 1933, BLISS 1938, BLISS and HANSON 1939).

Analysis of variance and covariance has been carried out on the individual values, and the tests of significance made by using variance ratios according to SNEDECOR (1938) and BONNIER and TEDIN (1940).

The material was divided into three main groups, according to concentration:

0.51—0.55 %	5	groups	29	animals
1.26 %	11	»	82	»
2.00—2.22 %	8	»	43	»

The analysis of covariance showed that the differences between these group means and between group regression coefficients were not significant ($P = 0.2$). This means that the differences in weight and concentration are eliminated by relating log lethal dose to mg drug injected per min and 100 g, and the main groups of concentration have then been combined into one.

The regression line has the general form

$$\log Y = \log \bar{y} + b(x - \bar{x}) \quad \ldots \quad (1)$$

where $\log Y$ = log lethal dose of a value on the regr. line,
$\log \bar{y}$ = mean of log lethal doses found,
b = regression coefficient,
x = mg drug injected per min and 100 g,
$\bar{x}$ = mean of x-values.

When the log lethal doses and the injection rate are expressed in mg fol. dig. int. as stated above, the regression line of the Digisolvin series becomes

$$\log Y = 1.1819 + 0.06735\,(x - 1.6427) \quad \ldots \quad (2)$$
$$\log Y = 1.0720 + 0.06735\,x \quad \ldots \quad (3)$$

Correlation coefficient $(r) = 0.7457$ ($P < 0.001$).

Group means of actual or found lethal doses and regr. line are illustrated in fig. 3 A.

In order to test whether

1) the group means of the adjusted lethal doses show any departure from the regr. line beyond the exp. error = heterogeneity of the material, or

2) the adjusted log lethal doses are normally distributed, each actual value (y_{found}) was adjusted to the identical injection rate by means of the following formula (4):

$$\log y_{adjusted} = \log y_{found} - \log Y + \log y_c \quad \ldots \quad (4)$$

where $\log Y$ is calculated from formula (3) for each x-value and
$\log y_c$ is a chosen standard, *e. g.* the median lethal dose = $\log \bar{y}$, corresponding to LD 50, or in *this* case the log lethal dose corresponding to a rate of injection of 2.22 mg per min and 100 g = 1.221 = log 16.65 mg per 100 g, calculated by means of formula (3).

Ad 1. The actual and adjusted log lethal doses were divided into groups according to the original rates of injection (table 1 and 3).

The stand. dev. in col. 5 and 11 are as usual calculated as the square root of the mean square (col. 4 and 10). This being a logarithm, σ too becomes a log, and its antilog the stand. dev. in percentage of the

Table 3.
Digisolvin.

Source of Variation	Actual Values						Adjusted Values					
	Degrees of Freedom	$S\ (y-\bar{y})^2$	Mean Square	Stand. Deviation			Degrees of Freedom	$S\ (y-Y)^2$	Mean Square	Stand. Deviation		
				$\log \sigma$	Per cent of Average	mg fol. dig.				$\log \sigma$	Per cent of Average	mg fol. dig.
1	2	3	4	5	6	7	8	9	10	11	12	13
Rates of injection	23	0.697117	0.030309	0.1741	40.1	6.76	22	0.125348	0.005698	0.0765	17.4	2.89
Deviations within groups (error) . . .	130	0.364501	0.002804	0.0520	12.2	2.03	129	0.345880	0.002681	0.0518	11.9	1.97
Total	153	1.061618	0.006939	0.0832	19.2	3.19	152	0.471228	0.003100	0.0557	12.8	2.15

Test of significance:

Variance ratio $\frac{0.030309}{0.002804} = 10.8^{***}$ (Df 23/130 P = 0.001) $\frac{0.005698}{0.002681} = 2.13^{**}$ (Df 22/129 P = 0.01)

average; on account of its conversion from logarithms its value is larger when positive than negative. When σ is small as compared to the average, this difference can be approximated according to COCHRAN (1938), and expressed in percentage of the mean:

$$\text{Stand. dev.}_{\text{percentage}} = \ln 10 \times \log \text{stand. dev.} \quad . \quad . \quad . \quad (5)$$

$$\sigma_g = 2.3026 \times \log \sigma \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

Col. 6 and 12 are calculated according to formula (6), and col. 7 and 13 expressed in mg fol. dig. int., the average being antilog 1.221 = = 16.65 mg per 100 g.

The group means differed significantly from each other, even after adjustment, indicating a heterogeneity of the material.

Ad 2. BLISS (1938) has given a method for converting mortality curves to straight lines by calculating percentage mortality and transforming them to probits, which permits calculation of LD 50 and the stand. dev. The same procedure can be applied to lethal doses (BLISS and HANSON 1939). The log lethal doses are divided into groups according to size, the groups expressed in percentage of the total, *i. e.* giving percentage mortality, and these converted to probits. If the log lethal doses are normally distributed, the converted mortality curve becomes rectilinear, the reciprocal of its slope giving the stand. dev.

This has been applied to the adjusted values of the Digisolvin series, and the results are to be found in fig. 5 A. The agreement with expectation is a support for the assumption that the lethal doses are normally

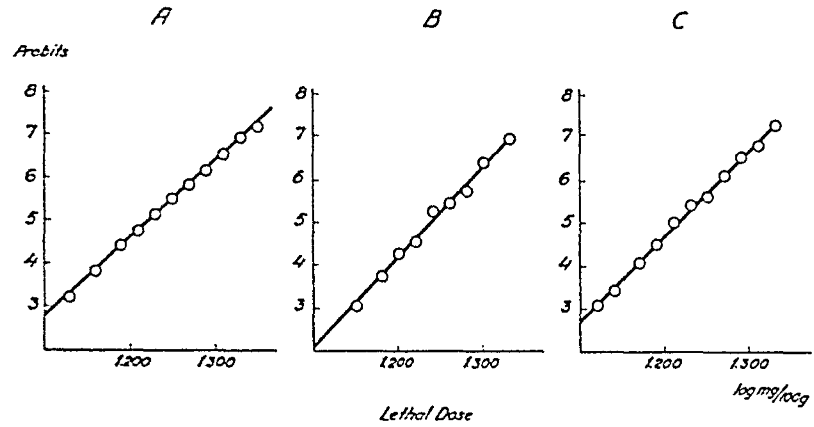

Fig. 5.

A. Digisolvin. B. Fol. dig. nat. stand. (K. I.). C. Fol. dig. nat. stand. (S. P. L.). Percentage mortality of adjusted log lethal doses, mg fol. dig. int. pr 100 g, transformed to probits and plotted against log lethal doses in mg pr 100 g. Mortality line by graphical construction.

distributed and that by applying the regression equation the identical results are obtained as when using the mortality curve according to Bliss et al., both with regard to the size of the median lethal dose and to the stand. dev.

B. Fol. Dig. Nat. Stand. (K. I.).

The test being expressed in mg fol. dig. int. per 100 g and the rate of injection in mg fol. dig. int. per min and 100 g, the regression line of this series had the formula

$$\log Y = 1.2649 + 0.05463\ (x - 2.7355) \quad \ldots\ldots \quad (7)$$
$$\log Y = 1.1154 + 0.05463\ x \quad \ldots\ldots\ldots\ldots \quad (8)$$

Correlation coefficient $(r) = 0.8366$ $(P < 0.001)$.

The values have then been divided into the original groups, according to dates of testing — 10 groups with 38 animals —, and adjusted by means of formulae (4) and (8) to the identical rate of injection — 2.22 mg per min and 100 g — corresponding to a mean lethal dose in this series of antilog 1.237 = 17.26 mg per 100 g, calculated by formula (8). The group means and regr. line are illustrated in fig. 3 B, and the result of the analysis of variance in table 4.

Stand. dev. calculated as in A., the average here being antilog 1.237 = 17.26 mg fol. dig. int. per 100 g. The differences between the group means are not significant after adjustment.

The result of converting log lethal doses to probits according to Bliss et al. is illustrated in fig. 5 B. The values agree fairly well with expectation.

Table 4.

Fol. digit. nat. stand. (K.I.).

Source of Variation	Actual Values						Adjusted Values					
	Degrees of Freedom	$S(y-\bar{y})^2$	Mean Square	Stand. Deviation			Degrees of Freedom	$S(y-Y)^2$	Mean Square	Stand. Deviation		
				log σ	Per cent of Average	mg fol. dig.				log σ	Per cent of Average	mg fol. dig.
1	2	3	4	5	6	7	8	9	10	11	12	13
Rates of injection	9	0.176843	0.019639	0.1401	32.3	5.57	8	0.027451	0.003050	0.0552	12.7	2.20
Deviations within groups (error) . . .	28	0.083385	0.002978	0.0546	12.6	2.17	27	0.050634	0.001875	0.0433	10.0	1.72
Total	37	0.260228	0.007033	0.0839	19.3	3.33	36	0.078085	0.002169	0.0466	10.7	1.85

Test of significance:

Variance ratio $\frac{0.01964}{0.00298} = 6.59^{***}$ (Df 9/28 P = 0.001) ‖ $\frac{0.003050}{0.001875} = 1.63$ (Df 8/27 P = 0.2)

C. Fol. Dig. Nat. Stand. (S. P. L.).

The test was expressed in mg fol. dig. int. per 100 g and the injection rate in mg per min and 100 g. The regression line for the whole series — 16 groups with altogether 85 animals — had the formula

$$\log Y = 1.2242 + 0.08406\,(x - 2.3486) \quad \ldots\ldots \quad (9)$$

$$\log Y = 1.0268 + 0.08406\,x \quad \ldots\ldots\ldots\ldots \quad (10)$$

Correlation coefficient (r) = 0.8321 ($P < 0.001$).

Actual and adjusted values, according to formulae (4) and (10), have then been divided into the original groups, according to dates of test, the average being antilog 1.214 = 16.37 mg fol. dig. int. per 100 g, corresponding to a rate of injection of 2.22 mg per min and 100 g (table 5). Group means and regr. line are illustrated in fig. 3 C.

The differences between the actual group means were highly significant, different rates of injection being used, and even after adjustment to the identical injection rate a slight difference was to be found.

The result of the transformation of log lethal doses of this series to probits is given in fig. 5 C, the values agreeing well with expectation.

Table 5.

Fol. digit. nat. stand. (SPL).

Source of Variation	Actual Values						Adjusted Values					
	Degrees of Freedom	$S(y-\bar{y})^2$	Mean Square	Stand. Deviation			Degrees of Freedom	$S(y-\bar{y})^2$	Mean Square	Stand. Deviation		
				log σ	Per cent of Average	mg fol. dig.				log σ	Per cent of Average	mg fol. dig.
1	2	3	4	5	6	7	8	9	10	11	12	13
Rates of injection	15	0.517469	0.034498	0.1857	42.8	7.00	14	0.058588	0.004185	0.0647	14.9	2.44
Deviations within groups (error) . . .	69	0.124199	0.001800	0.0424	9.8	1.60	68	0.139686	0.002054	0.0453	10.4	1.71
Total	84	0.641668	0.007639	0.0874	20.1	3.29	83	0.198274	0.002388	0.0489	11.3	1.84

Test of significance:

Variance ratio $\frac{0.03450}{0.00180} = 19.2^{***}$ (Df 15/69 P = 0.001) || $\frac{0.004185}{0.002054} = 2.04^{*}$ (Df 14/68 P = 0.05)

Summary.

The guinea-pig method for standardization of digitalis preparations (Knaffl-Lenz 1926) has been tested with various concentrations, rates of injection and weights.

1. The lethal dose (or log lethal dose) varies proportionately to the quantity of drug injected per min and 100 g. The variations may amount to max. 44 %, on an average 20 %, when following Knaffl-Lenz' original directions. When keeping standard conditions, or adjusting the lethal doses to a constant rate of injection by means of the calculated regression equation, the standard average deviation is 10—12 %.

2. When determined at *repeated* intervals under standard conditions the *guinea-pig unit* shows an average variation of 5—10 %.

3. The potency of digitalis preparations should be expressed in international units or g of a standard preparation and be uniformly tested on the same stock of animals as the standard. Under these conditions the guinea-pig unit has a stand. error of 3—5 %, 5—6 animals being used for each test, and the guinea-pig method seems to be a fairly good method of standardization.

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From the Zoophysiological Laboratory, Copenhagen.

The Blood-Brain-Barrier in Some Freshwater Teleosts.

By

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(Received 8 May 1942.)

It is a wellknown fact, that the passage of several substances from the blood to the central nervous system is very slow in mammals; it is usually said, that a blood-brain-barrier is present in these animals (references by BROMAN).

Two methods have been used to demonstrate the presence of such a barrier. The first is injection of certain vital dyes outside the central nervous system, e. g. intravenous or subcutaneous, i. e. "paraneural".

Hereby all tissues, except the brain and the medulla spinalis, may be coloured. In the brain, only the peculiar glandlike formations, called plexus choroideus, become strongly stained.

This procedure was designed by GOLDMANN (1913) and has been used lately by BROMAN (1940).

The second method has been to analyse the cerebro-spinal fluid after (paraneural) ingestion of different substances. It has been extensively applied by modern authors (see e. g. STERN (1921)).

As it was not known, whether similar mechanisms are present in other vertebrates, I undertook, on the suggestion of prof. KROGH, to investigate the state of teleosts in this connection.

The structures belonging to the central nervous system are in fishes widely different from those in higher vertebrates. Of interest for the present problem are mainly the meninges and the choroid plexuses.

The Meninges.

The classical view of the meninges in teleosts is that the meninx primitiva of the young larva is split up into an endomeninx close to the central nervous system, and an ectomeninx bordering on the cranium and corresponding to the dura mater of higher vertebrates. The large space between these two membranes is filled with very loose connective and fatty tissue. According to most modern authors (KAPPERS (1925), VAN GELDERN (1925)) there is present in most (possibly all) teleostean fishes, a third membrane between the two mentioned, and VAN GELDERN has further shown,

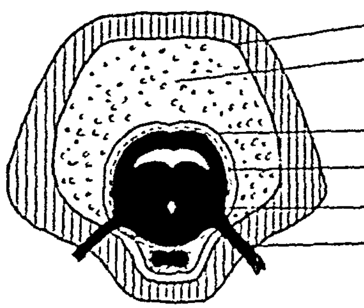

Fig. 1. Cross-section of the skull of a cyprinoid fish (midbrain-region, schematic). From SAGEMEHL, modified.)

that it is derived from the endomeninx. This lamina externa of the endomeninx as VAN GELDERN calls it, is built up of fibrous tissue and has no resemblance to the arachnoidea of the amniote vertebrates.

The Choroid Plexuses.

The anterior choroid plexus of the teleostean fish is a transparent, very thin and fragile membrane covering the anterior ventricle, at bottom of which the basal ganglions are situated. (Fig. 2.) It corresponds to the pallium of other vertebrates (it is in fact often called pallium). The posterior choroid plexus is a formation covering the fourth ventricle. It is in most species considerably smaller than the anterior plexus and was not described untill in the second decade of this century (COUPIN (1924)). The choroid formations are not folded as in other vertebrates (including cyclostomes, selachians and ganoids) but consist of a single layer of high cylindrical ciliated cells with sharp cell-borders; outside

these a richly vascularised layer of connective tissue (that is directly connected with the adipose meningeal tissue) is found.

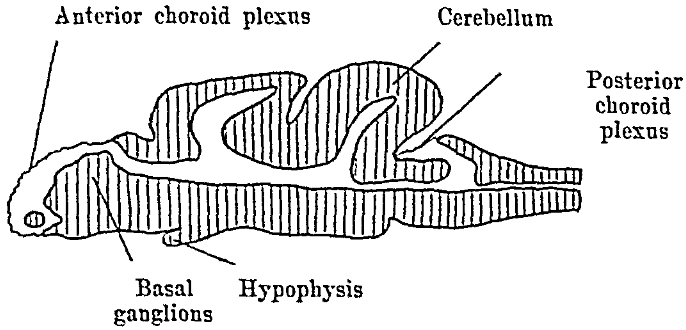

Fig. 2. Longitudinal section of teleostean brain, schematic. (From COUPIN, modified.)

The Method Used.

It was decided to use T. BROMAN's staining-method. According to his experiences on mammals, Trypanblue, (an acid azo-compound of mol.-weight about 900) is the most suitable dye, and it was used without further investigation.

In order to obtain a distinct colouration of the tissues two ways have been tried, either to maintain a high concentration of the dyestuff in the blood for a reasonably short period, or to use a lower concentration during a correspondingly longer time. A high concentration in the blood can be reached only by injection directly into the vascular system, and in the animals here used the only practicable way is injection into the heart. The blood-volume of fishes however is very small (about 1.5 % of the weight) and only a very limited amount of extra fluid is tolerated. Therefore, as Trypanblue is only sparingly soluble, several small injections would have to be performed, involving considerable technical difficulties.

A prolonged diffusion of the dye into the bloodstream is obtained by intraperitoneal injection. Larger amounts of liquid are tolerated in the peritoneal cavity, and the disappearing fluid is easily replaced by renewed injection.

Experimental Procedure.

The experimental animals were roach (Leuciscus rutilus), bream (Abramis brama), perch (Perca fluviatilis) and tench (Tinca vulgaris) weighing from 200—700 gr. They were caught in nets in the lake Fureso and kept in large aereated tanks with continually renewed tap-water. They were not fed, as their stay in the laboratory never exceeded 2 weeks.

The injection-fluid was prepared by dissolving 2 % Trypanblue in warm 0.7 % NaCl-solution. The solution obtained is supersaturated; by standing for a longer period a deposit of the dye is formed, it was therefore allways filtered before use.

The fishes to be injected directly into the blood stream, were narcotized with urethane, (they were placed in a small aquarium containing a 1 % urethane-solution, until swimming motions had ceased). For the operation; they were fixed in a special fishholder, the ventral side upwards, and the injection performed in the heart-ventricle. If the injection is performed correctly, a dark blue colour is seen to spread immediately over the gills. This procedure requires some practice, as the position of the heart is not very favourable for this kind of operation. In this way about 3 cc/kg can be injected at a time; the rate of injection did not exceed 0.2 cc/min. If larger amounts are injected, the animals will not recover from the narcosis, and by the post mortem examination they are found to have the heart dilated.

For the intraperitoneal injections narcosis is not necessary, the fish is held in a wet cloth when the injection is made. In this way 6—8 cc/kg can be given without seriously affecting the animal. In the best experiments this dose was given on two succeeding days; 12 hours after the second injection the animal is generally rather weak, it is killed (with urethane), and the vascular system is perfused from the ventral aorta with 0.7 % NaCl-solution under a pressure of 50—60 cm water. When the outflowing fluid is practically colourless, perfusion is stopped and samples of different organs taken out and placed in 4 % formaline. It was not found necessary to fix by injection and examine the tissues immediately (as does BROMAN); the staining is fairly stable and microscopic examination after fixation for 24 hours was found to give reliable results.

Sections for histological examination were made on a Jung freezing-microtome. Generally sections of 10 μ were found to give satisfaction; in some cases of faint staining (muscle) thick slices (200 μ) was used.

As it was nearly always found, that the loose, fatty tissue in the large (subdural) space inside the cranium was definitely coloured, some experiments (mostly with breams) were made with injections of the dyestuff directly into this cavity. Two small holes were bored in the skull of the narcotized fish; one in the region of the lobi olfactorii, the other just above the cerebellum. In one of the holes a fine cannula was inserted, and very cautiously a few drops of dyestuff solution was injected, whereupon the holes were sealed with wax. The animal was killed after the lapse of 4—5 hours and the brain examined.

Results.

In all experiments with intraperitoneal or intracardial injection, no trace of blue colour was detected in the nervous tissue of the brain. (12 successful experiments of the first, 4 of the second kind were made). The choroid plexuses, when observed, were definitely

blue, as was also the nearly fluid tissue of the subdural space. In some cases the hypophysis was examined and found to be faintly stained.

The gut (especially the submucosa) and the liver were strongly coloured as was also the kidney tissue. Striated muscle (from the pectoral fins) was only stained in longlasting experiments, probably a consequence of the poor capillarisation (KROGH, 1929).

Small peripheral nerves (from the lateral-line organ) were distinctly blue, whereas the large spinal nerves were only superficially stained.

The experiments with subdural application of Trypanblue gave the following results. In two cases the subdural tissue was strongly coloured, while the brain showed no trace of colour. In several experiments the brain was locally stained to a depth of about 1 mm; it may safely be assumed, that this staining was due to damage done to the fragile endomeningeal tissue by the injection

Conclusions.

The central nervous system is also in fishes abundantly supplied with bloodvessels, (as was clearly seen on some sections made of unperfused, dye-containing brains). It may therefore from the preceeding description be concluded, that some obstacle to the penetration of the dye into the central nervous system (i. e. a blood-brain-barrier) is present. That the results are not due to insufficient concentration of Trypanblue in the blood is seen from the fact, that all other tissues (including peripheral nerves), were distinctly stained. The possibility that the azo-dye might be decolourized by the brain tissue has been considered by BROMAN (1940). He placed brain tissue of a cat together with a small amount of Trypanblue in Thunberg-tubes under anaerobic conditions for several hours, but did not notice any decoloration.

The present investigation allows no conclusions regarding the localisation of the barrier.

Summary.

By paraneural injection of Trypanblue the presence of a blood-brain-barrier has been demonstrated in some common freshwater teleosts.

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Der Eiweisstoffwechsel der Gewebezellen in vitro.

1. Die Wirkung homologer und heterologer, durch Pepsin und Erepsin abgebauter Eiweisse.

Von

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(Eingegangen am 11. Mai 1942.)

Bei Abwesenheit von Abbauprodukten des Eiweisses sind tierische Gewebezellen nicht imstande, ihr Leben in einem aus homologem Bluteiweiss bestehenden Züchtungsmedium fortzusetzen (FISCHER 1941). Wird einem Plasmamedium, das durch Dialyse insuffizient gemacht worden ist, eine geeignete Mischung der wichtigen Aminosäuren zugesetzt, so bleiben die Gewebezellen nicht nur am Leben, sondern sie können sogar einiges Wachstum entfalten. Kochsäfte gewisser Organe, z. B. der Niere, haben eine weit grössere Wirkung und können das insuffiziente Medium beinahe ebensogut wie das nicht dialysierte Plasma komplettieren (FISCHER und ASTRUP 1942). Der Nierenkochsaft enthält also Stoffe, die für das Leben der Zellen wichtig sind und die in der Aminosäuremischung fehlen. Um zu entscheiden, ob die mehr oder weniger hochmolekularen Eiweissabbauprodukte für die gute Komplettierungsfähigkeit des Nierenkochsaftes gegenüber einer Mischung von reinen Aminosäuren verantwortlich sind, untersuchten wir, wie weit die Zellen Polypeptiden ausnutzen können. Eine sichere Methode zur Isolierung etwa vorhandener Polypeptiden aus einem Nierenkochsaft oder dem Blutserum gibt es leider nicht. Wir wählten dazu

das Verfahren, dialysiertes Serum mit Pepsin zu verdauen. Hierbei bekam man Mischungen von höheren und niederen Peptiden, die entweder keine oder doch nur geringe Mengen von freien Aminosäuren enthielten.

Nachdem festgestellt worden ist, dass die Gewebezellen Polypeptide ausnutzen, haben die Untersuchungen auch eine andere Bedeutung bekommen. Aus einer früheren Arbeit (FISCHER 1941), ging hervor, dass die Aminosäuremischungen nur dann günstig wirkten, wenn die einzelnen Säuren des Gemisches in den gleichen einfachen ganzzahligen Verhältnissen zu einander stehen, wie sie im Fibrin enthalten sind (BERGMANN und NIEMANN 1936). Die Wirkung blieb völlig aus, oder war höchstens sehr gering, wenn das Gemisch aus gleichen Gewichtsmengen sämtlicher Säuren bestand. Diese Untersuchungen gaben zu der Annahme Anlass, dass der Organismus während des Fastens nicht nur eine stets gleiche Konzentration des Aminosäurespiegels aufrecht zu halten sucht, sondern auch ein ganz bestimmtes Verhältnis der Aminosäuren untereinander, das für jede einzelne Tierart charakteristisch wäre.

Eine erweiterte Kenntnis vom Eiweisstoffwechsel der Gewebezellen würde von allgemeiner physiologischer Bedeutung sein und könnte möglicherweise einen Einblick in den Konfigurationsunterschied analoger Eiweisse verschiedener Tierarten eröffnen. Es liegt nahe, zu untersuchen, ob ein beliebiger Gewebezelltyp einer und derselben Tierart in gleichem Ausmasse Polypeptide, d. h. durch Pepsin verdaute Sera artfremder Tiere, ausnutzen kann. Ebenso wichtig würde es sein, zu erfahren, ob Polypeptide aus artfremdem Eiweiss in gleichem Ausmass von sämtlichen Gewebezelltypen einer Tierart ausgenutzt werden. Derartige Untersuchungen würden gleichzeitig Auskunft darüber geben, ob grundsätzliche Unterschiede im Eiweisstoffwechsel verschiedener Zelltypen bestehen. In qualitativer Hinsicht sind die Aminosäuren, die in analoge Eiweisse, z. B. Fibrin, verschiedener Tierarten eingebaut sind, wohl die gleichen. Eine entscheidende Rolle spielt dagegen die Art und Weise, wie die einzelnen Säuren sich in der Peptidkette periodisch wiederholen und auf diese Weise gewissermassen ein Muster bilden.

Im folgenden werden Vergleichsuntersuchungen über die komplettierende Wirkung pepsin- und erepsinverdauter dialysierter homologer und heterologer Eiweisse auf das insuffiziente Züchtungsmedium beschrieben.

1.
Technik.

In einer vorhergehenden Arbeit (FISCHER 1941) ist die Technik für die Durchführung einer aseptischen Dialyse von Blutplasma und Serum ausführlich beschrieben worden. Später haben Untersuchungen, (FISCHER und ASTRUP 1942) ergeben, dass es erforderlich war, eine weitergehende Dialyse auszuführen. Gegenwärtig lassen wir die Dialyse in zwei Phasen vor sich gehen.

20 ml Heparin-Plasma bzw. Serum werden in der Kälte 4 Tage gegen 1.5 l Glukose-Ringer dialysiert. Hiernach wird die Membran mit dem Inhalt in einen neuen Kolben mit 1.5 l Glukose-Ringer übertragen, wo sie weitere 4 Tage steht. Dadurch wird die Verdünnung der dialysierbaren Stoffe auf 1/5625 (gegen die frühere von 1/75) gebracht, was ausreichend ist, um eine Fortsetzung des Lebens der Zellen in diesem Medium auszuschliessen. Nach beendigter Dialyse wird, des Bikarbonats wegen, gewöhnlich 25 % Tyrodelösung der Eiweisslösung zugesetzt.

Um der Gerinnung des Hühnerplasmas in der Membran vorzubeugen, wurde wie früher 2 ml 0.5 pro mille Heparin (Aktivität K = 1.4, ASTRUP (1938)) zugefügt. In einigen Fällen haben wir, um den Zusatz dieses fremden Stoffes zu vermeiden, das Plasma gegen eine kalziumfreie Glukose-Ringer dialysiert. Dieses Verfahren hat u. a. den Vorteil, dass die Gerinnung mit dem dialysierten Gewebeextrakt nach der Rekalzifizierung sehr viel schneller vor sich geht als mit Heparin.

Für die Versuche wurden wie früher periostale Fibroblasten aus dem Os frontale des 14tägigen Hühnerembryos benutzt. Ausserdem sind Fibroblasten aus Herz und Leber des Hühnerembryos in der 7.—20. Passage verwandt worden. Die Kulturen für die Versuche wurden aus zwei-, seltener aus dreitägigen Deckglaskulturen gewählt.

Die eine Hälfte einer Kultur diente als Kontrolle der andern. Täglich wurden die Umrisse der Kulturen bei 19facher linearer Vergrösserung aufgezeichnet und mit dem Planimeter gemessen. Gleichzeitig wurde sorgfältig mikroskopiert und die Ergebnisse auf die entsprechende Umrisszeichnung eingetragen. Die Auswertung der Flächenausbreitung (Wachstum) wurde nach der Formel $\frac{B - A}{A}$ vorgenommen, wobei A die Fläche der Kontroll- bzw. Experimentkultur beim Versuchsbeginn, B die Fläche nach Ablauf einer bestimmten Zeit ist.

Die Versuche wurden in folgender Weise ausgeführt: 0.5 ml zweifach dialysiertes Plasma, das zu 25 % Tyrodelösung enthielt, wird in die CARREL-Flaschen eingebracht; 1.0 ml Ringerlösung wird zugesetzt. Während das Medium noch flüssig ist, wird 0.1 ml der Lösung mit den betreffenden Stoffen, deren Wirkung untersucht werden soll, hinzugegeben. Die Erfahrungen haben uns gelehrt, dass der Ausgleich der betreffenden Stoffe zwischen flüssiger und fester Phase des Mediums eine gewisse Zeit in Anspruch nimmt. Wird deshalb nicht

sofort etwas davon dem Plasmamedium zugesetzt, so kann es geschehen, dass das Gewebestück zu Grunde geht, bevor ein Austausch stattgefunden hat. Hiernach wird ein Tropfen dialysierter Embryonalextrakt zugefügt und das Gewebestück in die Flasche eingebracht. Die Flaschen bleiben abgestellt, bis die Gerinnung des Plasmas stattgefunden hat. Wenn dies eingetreten ist, wird 0.5 ml dialysiertes Hühnerserum dem Gerinnsel überschichtet, und dazu wird 0.2 ml derselben Lösung des für die Untersuchung in Frage kommenden Stoffes beigegeben. Man muss sorgfältig darauf achten, dass die flüssige Phase die ganze Plasmaoberfläche vollständig benetzt. Die Flaschen werden in gewöhnlicher Weise mit einem Gummihütchen zugemacht. Die Umrisse der Gewebstücke werden aufgezeichnet und die Kulturen in den Brutschrank bei 39° gesetzt. Alle 3—4 Tage wird das flüssige Nährmedium abgesaugt. Die Kulturen werden 10—15 Minuten mit Tyrodelösung, die vorher mit einer Gasmischung von 80 % O_2, 8 % CO_2 und 12 % N_2 durchlüftet ist, gewaschen. Hierdurch wird die Tyrode auf etwa pH 7 eingestellt und gleichzeitig mit genügend O_2 gesättigt. Nach Absaugung der Waschflüssigkeit wird wieder 0.5 ml dialysiertes Serum und die gleiche Menge der in Frage stehenden Stoffe hinzugefügt. Als Sicherung gegen Zufälligkeiten wurden mehrere Versuche und Kontrollen zu gleicher Zeit ausgeführt.

Um mit Pepsin und Erepsin Abbauprodukte der Proteinlösungen darzustellen, sind wir in folgender Weise vorgegangen: Die verwendeten Sera wurden alle frisch hergestellt, durch einen Seitzfilter sterilfiltriert und schliesslich steril dialysiert. 20 ml dialysiertes Serum wurden mit destilliertem Wasser bis 50 ml aufgefüllt und mit HCl bis pH 3 sauer gemacht. 100 mg käufliches Pepsin (Aktivität 1—3,000) wurden 24 Stunden in saurer Lösung dialysiert, um dadurch das Hineingeraten etwaiger Abbauprodukte bei den Versuchen zu verhindern. Vorher wurde zur Sicherheit festgestellt, dass das Pepsin nach der Dialyse seine volle Aktivität behalten hat. Die Verdauung ging unter Zusatz von ein wenig Chloroform bei 38° 24 Stunden lang vor sich. Darauf wurde die Lösung neutralisiert, gekocht, filtriert und schliesslich im Autoklaven sterilisiert. Eine Probe für die N-Bestimmung wurde entnommen und die Lösung auf eine passende Konzentration die für sämtliche Lösungen gleich war, eingestellt. Kontrollen mit Pepsin allein wurden ebenfalls hergestellt.

Für den weiteren Abbau mit Erepsin wurde ein Teil des vorher mit Pepsin abgebauten Eiweisses entnommen. Das Erepsin wurde ganz wie das Pepsin 24 Stunden lang vorher dialysiert; es behielt gleichfalls seine volle Wirkung nach der Dialyse.

1 g Erepsin (Schuchardt) wurde in 20 ml physiol. Kochsalz gelöst. Hiervon wurde 1.5 ml (entspr. etwa 75 mg Enzym) zu 25 ml pepsinverdautem Dialyseserum, das mit NaOH schwach alkalisch gemacht war, zugesetzt. Mit ein wenig Chloroform wurde die Lösung 48 Stunden bei 38° abgestellt. Als Kontrolle für das Enzym wurde eine entsprechende Lösung von dialysiertem Pepsin in Wasser unter Hinzufügung des Erepsins ebenfalls bei 38° verdaut. Die Mengenverhältnisse waren die gleichen wie bei der Verdauung der Proteine.

Als Beispiel für den Umfang des Abbaus durch Erepsin führen wir die folgenden Versuche an:

Hühnerserum (13/12) Total-N (Kjeldahl) = 1.05 mg N/ml. Amino-N vor der Verdauung mit Erepsin = 0.191 mg N/ml; nach Abbau mit Erepsin = 0.604 mg N/ml. Amino-N der Erepsinlösung allein = 0.196 mg N/ml.

Ochsenserum (2028 P) Total-N (Kjeldahl) = 1.66 mg N/ml. Amino-N vor dem Abbau mit Erepsin = 0.203 mg/ml; nach Erepsin = 0.934 mg N/ml. Amino-N der Erepsinlösung allein = 0.196 mg N/ml.

Die Präparate, die für die Versuche verwendet wurden, haben die Konzentration von 70 und 35 mg% N, d. h. die Konzentration war, auf das Züchtungsmedium umgerechnet, 7 bzw. 3.5 mg% N.

2.

Experimentelles.

Schon früher ist gezeigt worden (FISCHER 1941), dass Gewebezellen in dialysiertem Blutplasma und Serum sehr schnell eingehen, jedoch bei Zusatz geringer Mengen eines durch Trypsin abgebauten Hühnerserums am Leben bleiben. Diese Tatsachen geben uns zwei wichtige Auskünfte: 1.) Die Gewebezellen sind nicht imstande, jedenfalls nicht unmittelbar, die Eiweisse im Medium zu zerlegen; 2.) die Eiweissabbauprodukte spielen eine entscheidende Rolle für den Stoffwechsel der Zellen. Hierbei muss jedoch stets im Auge behalten werden, dass die mit Trypsin abgebauten Eiweisse keine einheitlichen Produkte sondern ein Gemisch von Aminosäuren und Peptiden bilden.

Wird ein dialysiertes Hühnerserum, das durch Pepsin abgebaut ist (89.2) den Kulturen zugesetzt, so fangen die Zellen zu wachsen an, bisweilen ebenso gut wie in einem nicht dialysierten Medium. Das Wachstum kann recht ansehnliche Grössen erreichen, hört aber meistens wieder auf, während die Zellen noch lange Zeit am Leben bleiben und in morphologischer Hinsicht alle Zeichen von gesunden Zustand aufweisen (3661). Verglichen mit der Wirkung von Gemischen aus reinen Aminosäuren ist die Wirkung der durch Pepsin abgebauten Eiweisse weit grösser. Setzt man ausserdem den Kulturen dialysierten Embryonalextrakt hinzu, so können sie ganz ansehnliche Grössen erreichen (3845—50). Fig. 1 gibt die Wachstumskurve eines solchen Versuches an. Eine gleiche wachstumsfördernde Wirkung besass auch ein aus Kalbsembryonen isoliertes Nukleoproteid, (FISCHER 1941).

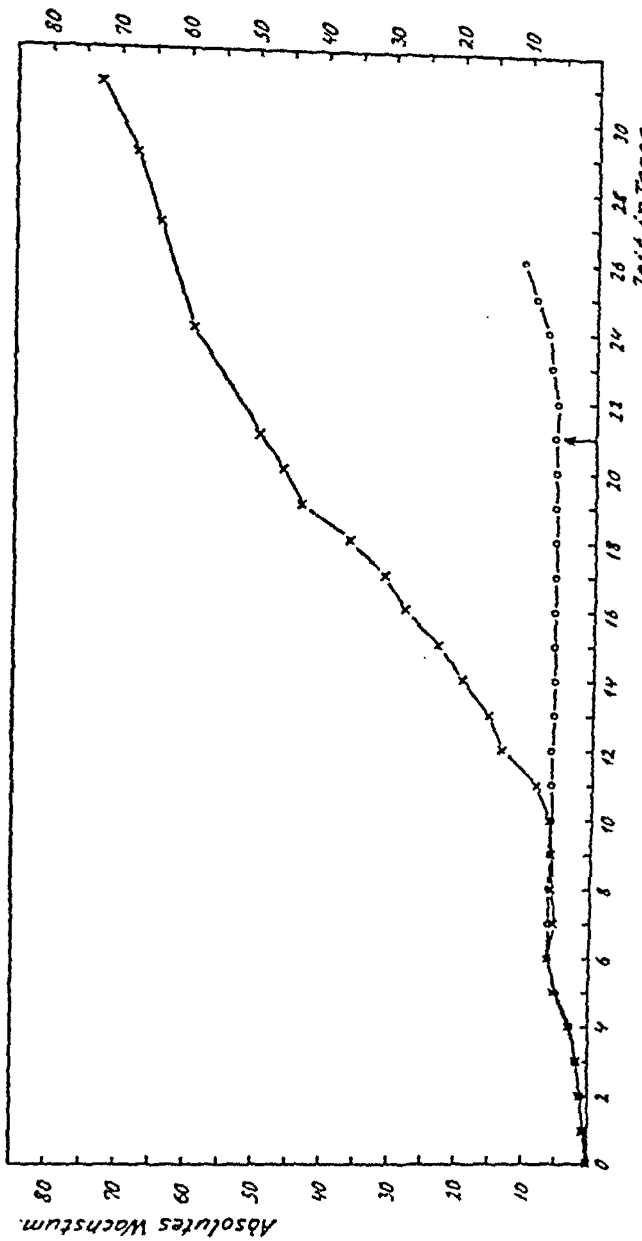

Fig. 1. (3847) Die Kurven geben das Wachstum zweier Kulturhälften von periostalen Fibroblasten in einem dialysierten Züchtungsmedium mit Zusatz von pepsinverdautem homologen, dialysiertem Serum an. ×——× mit Zusatz von dialysiertem Embryonalextrakt; O——O die Kontrolle ohne Extrakt. An der Stelle des Pfeiles wurde nichtdialysiertes Serum und Embryonalextrakt zugegeben, um festzustellen, ob die Kultur am Leben war. Als Folge hiervon der Wachstumsanstieg.

Man ersieht also daraus, dass die hochmolekularen Polypeptide aus irgendeinem Grunde in stärkerem Umfang als eine Mischung der wichtigen Aminosäuren von den Gewebezellen ausgenutzt werden können. Das kommt auch zum Ausdruck, wenn pepsinabgebautes Eiweiss weiter mit Erepsin abgebaut wird. Als Beispiel können wir folgenden Versuch angeben: Hühnerserum

(13/12) wurde zunächst mit Pepsin verdaut. Nach dem Kochen und Filtrieren wurde ein Teil hiervon mit Erepsin abgebaut. Den Kontrollkulturen wurde mit Pepsin, den Experimentkulturen mit Erepsin abgebautes Serum zugesetzt. Der Gehalt der Produkte in den Kulturen betrug 7 mg% N auf das ganze Medium umgerechnet. Die Versuche zeigten eindeutig ein viel grösseres Wachstum in den Kontrollen als in den Experimentkulturen mit den niedrigen Abbauprodukten. Das Wachstum fing gleich kräftig in den beiden Kulturhälften an. Während die Zellen der Kontrollkulturen klar und von gesundem Aussehen blieben, wurden die Zellen mit den niederen Produkten recht bald stark vakuolisiert und gingen unter Zerfall schnell ein (4205), Fig. 2.

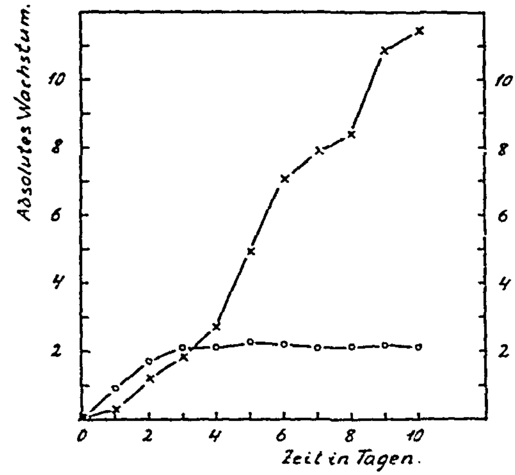

Fig. 2. (4205) Wachstumskurven zweier Kulturhälften von periostalen Fibroblasten in einem dialysierten Züchtungsmedium. ×- - -× mit Zusatz von pepsinverdautem homologen, dialysiertem Serum. O——O mit Zusatz von Pepsin-Erepsin-verdautem homologen Serum.

Diese Versuche zeigen deutlich, dass die Zellen besser die grösseren als die kleineren Eiweissspaltstücke ausnutzen. Gegen diese Versuche kann nicht, wie bei den Untersuchungen mit reinen Aminosäuren, der Einwand erhoben werden, dass eine oder mehrere der wichtigen Säuren fehlen könnten, denn es muss vorausgesetzt werden, dass den Serumeiweissen sämtliche wichtigen Aminosäuren eingebaut sind.

Vergleichende Untersuchungen sind auch über die Wirkung einer Reihe homologer und heterologer Eiweisse, die mit Pepsin und Pepsin-Erepsin abgebaut waren, angestellt worden. Es waren das: Dialysiertes Serum von Huhn, Schwein, Ochse, Kaninchen und Mensch; Ochsenfibrin (durch Defibrinierung von frischem rekalzifizierten Oxalatblut hergestellt); Eialbumin (6 mal umkristallisiert); Kasein (Hammarsten); Gelatine, Edestin und Gliadin.

Eine Versuchsreihe (3787) mit pepsinverdautem Serum von Huhn, Schwein, Kaninchen, Ochse und Mensch (7 mg% N im

Medium) ergab folgendes: 23 Kulturen mit pepsinverdautem Hühnerserum (2028 P) zeigten deutliches Wachstum, allerdings kein besonders grosses, während die Produkte der übrigen Sera garkeine oder nur bedeutend geringere Wirkung als die des homologen Serums zeigten. In einer anderen Versuchsreihe (3915) mit pepsinverdautem Dialyseserum von Huhn, Ochse und Schwein (2029 P) waren nur die Abbauprodukte des homologen Hühnerserums wirksam. Die übrigen Versuche fielen völlig negativ aus.

Bei Versuchen (4117) mit Präparaten aus Serum von Huhn, Schwein, Ochse (2029 P), Huhn (13/12), Kaninchen (2028 P), Kaninchenimmunserum (2031 P), Ochse und Mensch (2028 P) zeigten sich ebenfalls nur Wirkung von Serum aus Huhn und besonders gut aus Huhn (13/12); alle übrigen Produkte waren entweder ganz wirkungslos oder von nur schwacher und kurzdauernder Wirkung.

Präparate aus Ochsenfibrin waren bisweilen ziemlich aktiv (4425). Die Produkte aus den übrigen heterologen Seren zeigten ab und zu eine geringe komplettierende Wirkung, die aber nie auf die Höhe der entsprechenden Produkte aus dem homologen Serum kam. Wurde ausserdem dialysierter Embryonalextrakt den sämtlichen Kulturen zugegeben, so zeigten beinahe alle Kulturen mit Produkten der heterologen Sera etwas Wachstum. Das relative Verhalten der verschiedenen Produkte blieb aber dasselbe wie vorher. Mit Produkten aus dem homologen Serum wurde ein sehr grosses Wachstum erreicht, dagegen nur ein geringes und kurzdauerndes mit denen der heterologen Sera (4001; 4033; 4061—92).

Um sicherzustellen, dass die benutzten Enzyme keinerlei Anteil an den Versuchsergebnissen hatten, wurden Kontrolluntersuchungen über die komplettierende Wirkung der Enzyme unternommen. Dialysiertes Pepsin wurde in physiol. Kochsalz desselben Aziditätgrades wie im Verdauungsversuch gelöst und 24 Stunden bei 38° bebrütet. Ebenso wurde dialysiertes Erepsin in physiol. Kochsalz bei schwach basischer Reaktion gelöst, und weiter wurde Erepsin zu einer 24 Stunden lang bebrüteten Pepsinlösung nach Kochen und Neutralisation hinzugesetzt. Alle Lösungen wurden gleich lange Zeit bei 38° bebrütet, wie beim Versuch über Abbau der Eiweisse. Keine der Enzymlösungen war imstande, eine nennenswerte Wirkung auf die Zellen auszuüben (4383, 4483, 4493).

Ausser den erwähnten homologen und heterologen Seren wur-

den die Abbauprodukte einiger Eiweisse anderer Art untersucht. Hier zeigten sich ebenfalls Unterschiede in Bezug auf die Fähigkeit der Gewebezellen, die Produkte auszunutzen. Unter den wirksamsten der durch Pepsin abgebauten Eiweisse waren Eialbumin (4427), Edestin (4463) und zum Teil Gliadin (4433). Die Wirkung des Produktes aus Kasein war ziemlich gering, ganz wirkungslos war Gelatine, das ja an sich ein sehr unvollständiges Eiweiss ist, weil ihm ganz die Säuren Valin, Tyrosin, Tryptophan und Methionin fehlen.

Werden die pepsinabgebauten Eiweisse weiter mit Erepsin bis zu Aminosäuren verdaut, so verschieben sich die Verhältnisse etwas in der Richtung hin, dass die Produkte durchgehends wirksamer werden.

Ein Überblick über die Versuchsergebnisse (Tabelle 1) zeigt deutlich, dass ganz erhebliche Unterschiede in der grösseren oder

Tabelle 1.

Art des Eiweisses	Nr	Absolute Zunahme der Wachstumsfläche von osteogenen Fibroblasten[1]	
		Durch Pepsin abgebaut	Durch Erepsin abgebaut
Hühnerserum	4401	16.0	2.1
Ochsenserum	4413	0	4.8
Schweineserum	4301	0	2.8
Kaninchenserum	4801	3.9	—
Kaninchen-Immun Serum	4279	3.4	14.1
Menschenserum	4269	5.2	7.9
Ochsenfibrin	4421	0	18.7
Eialbumin kristallisiert	4427	11.0	11.0
Kasein (Hammarsten)	4347	0	24.2
Edestin	4351	2.7	23.9
Gliadin	4435	6.8	12.1
Gelatine	4339	0	0
Pepsin-Kontrolle	4487	1.7	—
Erepsin-Kontrolle	4484	—	1.7
Erepsin-Pepsin-Kontrolle	4488	—	1.3

[1] Nach der Formel $\frac{B-A}{A}$ in qcm bei 19facher linearer Vergrösserung.

geringeren Wirkung der Eiweisspaltstücke auf die Aufrechterhaltung von Leben und Wachstum der Gewebezellen in den insuffizienten Medien bestehen, — Unterschiede, die in keiner Weise einem Mangel an notwendigen Eiweissbausteinen zugeschrieben werden können sondern aus anderen Ursachen abzuleiten sind.

Während die Wirkung des pepsinverdauten Kaseins (Kasein P) im allgemeinen sehr gering ist, erwies sich das mit Erepsin abgebaute Kasein (Kasein E) als sehr aktiv (4347). Edestin E, das sich ebenso verhielt, ist bedeutend stärker aktiv als das Edestin P (4351). Was das Eialbumin betrifft, waren beide Präparate (Eialbumin P und E) beinahe gleich wirksam. Die Wirkung des Gliadin E erwies sich als grösser als die des Gliadin P (4435). Das pepsinverdaute Ochsenserum war, wie erwähnt, ganz unwirksam, wurde aber nach weiterem Abbau durch Erepsin deutlich, obgleich recht schwach, wirksam (4409). Gelatine P sowohl wie Gelatine E waren beide unwirksam (4451). Die Abbauprodukte des homologen Hühnerserums verhielten sich umgekehrt, indem das Hühnerserum P bedeutend wirksamer war als Hühnerserum E.

In der Übersichtstabelle 1 wird versucht, die Ergebnisse der Untersuchungen zusammenzustellen. Leider ist es noch nicht möglich, die Versuchsergebnisse in absoluten Zahlen anzugeben, da es sich um ein grosses, buntes Material von individuell verschiedenen Kultureinheiten handelt. Die Zahlenwerte geben als Querschnitt durch sämtliche Versuche ein recht getreues Bild der wirklichen Verhältnisse. Zusammenfassend kann man sagen, dass die Zellen im allgemeinen *die höheren Abbauprodukte des homologen Eiweisses besser als die niederen ausnutzen.* Hinsichtlich der Abbauprodukte der *heterologen Eiweisse* ist es im allgemeinen umgekehrt, indem *die niederen Spaltstücke besser als die höheren ausgenutzt werden.* Diese Ergebnisse zeigen deutlich, dass der Artunterschied der Eiweisse sich auch in der Wirkung ihrer niederen Abbauprodukte auf die Gewebezellen geltend macht.

Auch in morphologischer Beziehung macht sich der Unterschied zwischen den höheren und den niederen Spaltstücken der Eiweisse bemerkbar. Die Gewebezellen in sämtlichen *pepsinverdauten heterologen* Eiweissen zeigen ein besonders charakteristisches Bild. Das Protoplasma der Zellen zerfiel in grössere oder kleinere Stückchen, die oval oder kreisrund erschienen. Das Protoplasma blieb ganz glasklar mit einer einzelnen grösseren oder

kleineren Vakuole, Fig. 3. Das Bild ist dem sehr ähnlich, das wir früher beobachtet haben, wenn die Zellen in einem sehr unvollständigen Gemisch von Aminosäuren, oder nur mit Cystin allein, lebten. Die mit Erepsin abgebauten heterologen Eiweisse zeigten

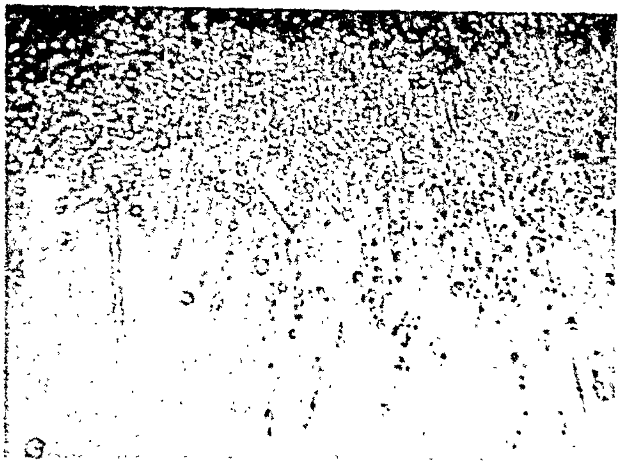

Fig. 3. (4267) Periostale Fibroblasten, lebend photographiert. (Vergr. 1 : 144). Mit pepsinverdautem Menschenserum.

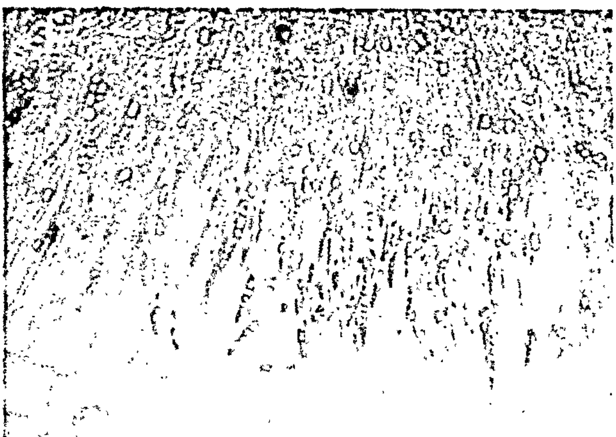

Fig. 4. (4276) Periostale Fibroblasten, lebend photographiert. (Vergr. 1 : 144). Mit Pepsin-Erepsin-verdautem Schweineserum.

sämtlich ein anderes Bild (Fig. 4). Die Zellen hatten ihre schlanke Spindelform behalten, zeigten aktive Pseudopodien, und das Protoplasma war meistens fein granuliert. Diese morphologischen Eigentümlichkeiten fehlten völlig in *pepsinverdautem homologen Serum.* Dieses charakteristische Bild deutet sehr darauf hin, dass

die Zellen unter einem Mangel an wichtigen Eiweissbausteinen leiden, die für die Synthese des Cytoplasmaeiweisses wichtig sind.

3.

Nachdem erkannt worden war, dass periostale Fibroblasten recht beträchtliche Unterschiede zeigen in Betrug auf ihre Fähigkeit, pepsinverdaute homologe und heterologe Eiweisse zu verwerten, war es naheliegend, festzustellen, ob auch andere Gewebezelltypen einer und derselben Tierart in gleicher Weise reagieren. Es besteht eine gewisse Wahrscheinlichkeit dafür, dass, so wie es strukturelle Unterschiede zwischen analogen Eiweissen verschiedener Tierarten gibt, auch Unterschiede im Bau der Eiweisse vorhanden sind, aus denen die verschiedenen Gewebezelltypen derselben Tierart bestehen. Die Unterschiede in den Eiweissen der einzelnen Tierarten sind derartig gross, dass sie sich immunologisch feststellen lassen. Dagegen sind die Unterschiede im Bauplan des Eiweisses verschiedener Zelltypen innerhalb derselben Tierart zu gering, um sich immunochemisch sicher nachweisen zu lassen.

Ist das Cytoplasma verschiedener Zelltypen einer Tierart nicht nach einem und demselben Muster aufgebaut, so dürfte das auch in der Wirkung ihrer synthetisierenden Enzyme zum Ausdruck kommen. Man würde dann wohl, wenn überhaupt, eher mit Hilfe der höheren Abbauprodukte, statt mit Mischungen von Aminosäuren, derartige Unterschiede im Eiweisstoffwechsel normaler und maligner Gewebezellen derselben Tierart nachweisen können. Zum Aufbau der Eiweisskörper der Zellen dienen sämtliche notwendigen Aminosäuren. Da es aber schliesslich auf den strukturellen Aufbau der Peptidkette ankommt und nicht auf die qualitative Zusammensetzung, wird man durch Variation in der Zusammensetzung der Aminosäuremischung nicht weiter kommen. Deshalb haben wir zunächst mit Untersuchungen über die Fähigkeit der Zellen, artfremde Peptide zu verwerten, begonnen.

Einige Tastversuche sind ausserdem mit Fibroblasten aus Leber- und Herzgewebe von Hühnerembryonen ausgeführt worden. Zunächst wurden diejenigen pepsinverdauten Eiweisse, die die grössten Unterschiede in der Wirkung auf die periostalen Fibroblasten aufwiesen, untersucht, nämlich Serum von Huhn, Ochse, Schwein; Ochsenfibrin, Eialbumin und Edestin.

Tabelle 2.

Art des Eiweisses (durch Pepsin abgebaut)	Nr	Absolute Zunahme der Wachstumsfläche	
		Herzfibroblasten	Leberfibroblasten
Hühnerserum	4443 4449	16.7	12.6
Ochsenserum	4444 4450	9.0	10.0
Schweineserum	4614 4626	2.6	2.2
Ochsenfibrin	4621 4631	5.9	0.7
Eialbumin	4548 4564	1.4	2.8
Edestin	4551	6.3	—

Tabelle 2 gibt eine Übersicht hierüber.

Während die periostalen Fibroblasten mit pepsinverdautem Ochsenserum nicht wachsen können, gab es in einigem Grade Wachstum bei Herzfibroblasten (4613). Leberfibroblasten besassen diese Fähigkeit beinahe in gleichem Umfang wie periostale Fibroblasten in homologen Produkten (4449), Fig. 5. Die Produkte des Schweineserums, die auf periostale Fibroblasten so gut wie unwirksam waren, erwiesen sich als einigermassen aktiv auf Herz- und etwas weniger auf Leberfibroblasten. Dasselbe gilt auch für Ochsenfibrin. Für die Aufstellung in der Tabelle 2 gilt dasselbe wie für Tabelle 1, nämlich dass die Zahlenwerte nur einen Durchschnittsausdruck für das ganze Versuchsmaterial darstellen.

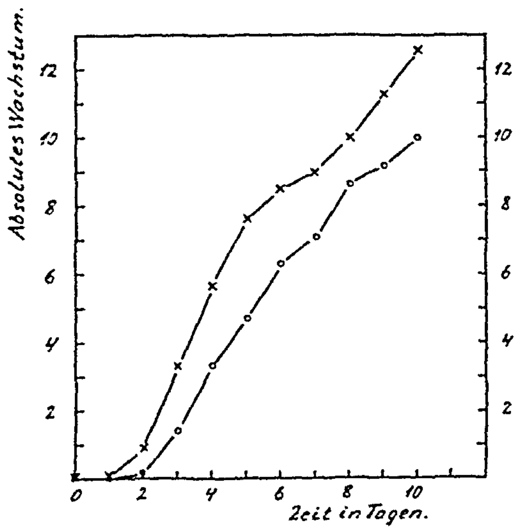

Fig. 5. (4449) Wachstumskurven zweier Kulturhälften von Fibroblasten aus Leber in einem dialysierten Züchtungsmedium. ×——× mit Zusatz von pepsinverdautem homologen Hühnerserum; O——O mit Zusatz von pepsinverdautem heterologen Ochsenserum.

4.
Besprechung.

Für die Untersuchungen über die Wirkung höherer Eiweissabbauprodukte wurden dialysierte Eiweisse, die mit Pepsin verdaut waren, verwendet. Es ist früher im allgemeinen angenommen worden, dass hierbei keine freien Aminosäuren entstehen. Inzwischen ist von verschiedenen Seiten angegeben, dass doch geringe Mengen von Aminosäuren nachgewiesen werden konnten (FELIX 1925, NORTHROP 1930, CALVERY und SCHOCK 1936, LIEBEN und LIEBER 1934). ABDERHALDEN (1941) ist jetzt auch der Meinung, dass ganz geringe Aminosäuremengen bei über lange Zeit ausgedehnter Verdauung im Reagenzglasversuch entstehen können. Bei unseren Versuchen ging die Verdauung 24 Stunden lang vor sich, was wohl kaum als besonders lange Dauer zu bezeichnen ist, so dass es schwerlich zur Bildung grösserer Mengen kommen konnte. Die Verdauung fand bei pH 3 statt, um zu verhindern, dass durch Säure ein zu weitgehender Abbau bewirkt wird.

Eine Lösung von höheren und niederen Polypeptiden aus homologem dialysierten Hühnerserum hebt den Mangelzustand des dialysierten Züchtungsmediums beinahe vollkommen auf. Die Gewebezellen bleiben am Leben und können auch bedeutendes Wachstum entfalten, das dem im Nierenkochsaft (FISCHER und ASTRUP 1942) an Grösse nicht nachsteht und sogar die Wirkung eines vollständigen aus reinen Aminosäuren bestehenden Gemisches übertrifft. Es ist deshalb als sehr wahrscheinlich anzunehmen, dass der Nierenkochsaft Polypeptide enthält. Die Frage, ob solche auch in der Blutbahn unter normalen Verhältnissen vorkommen, ist schon seit langem diskutiert worden. ABDERHALDEN (1931) hielt das Vorhandensein von Eiweissbausteinen, die eine Biuretreaktion geben, in der Blutbahn für ausgeschlossen. In der neuesten Zeit sind mehrere Untersuchungen über diese Frage ausgeführt worden, aus denen hervorgeht, dass Polypeptide doch einen normalen Bestandteil des Blutes bilden (CRISTOL und FOURCADE 1939, GODFRIED 1939). Der letztgenannte Verfasser gibt an, dass sie unter normalen Verhältnissen in einer Menge, die etwa 7 mg% N entspricht, vorkommen.

Die Untersuchungen haben gezeigt, dass Gewebezellen, die nicht genuines Eiweiss abbauen können (ob sie dazu imstande

sind, wenn niedere Bausteine anwesend sind, ist noch unbekannt), doch die mehr oder weniger hochmolekularen Abbauprodukte zu verwerten vermögen. Ob die Zellen sie ohne vorhergehenden Abbau in sich aufnehmen, oder ob sie zunächst abbauen, um sie dann wieder aufzubauen, wissen wir noch nicht. Aller Wahrscheinlichkeit nach ist jeder der beiden Vorgänge möglich. Die prompte Wirkung der homologen Abbauprodukte spricht für den ersten Weg, die schlechte Wirkung und die lange Wachstumslatenzzeit für den zweiten.

Die kleinen Eiweisspaltstücke sind meistens weit weniger wirksam als die grösseren, besonders wenn es sich um homologe Produkte handelt. Das durch Trypsin abgebaute homologe Dialyseserum erwies sich auch als recht wenig wirksam (Fischer 1941). Das Gleiche ging auch aus den Versuchen von Baker und Carrel (1928) hervor. Wie bekannt haben diese Verfasser beobachtet, dass die sogenannten Proteosen eine bedeutende wachstumsfördernde Wirkung auf die Gewebezellen ausüben. Die Untersuchungen wurden aber auf frischen, nicht-dialysierten Medien unternommen, und auf diesen übten die Proteosen eine weitere Erhöhung des auch ohne sie von selber stattfindenden Wachstums aus. Die Verhältnisse bei den Untersuchungen von Carrel und Baker waren also ganz andere als bei unseren eigenen. Auch die beiden eben genannten Verfasser geben übrigens an (1928), dass die wachstumsfördernde Wirkung bedeutend geringer wird, wenn die Produkte weiter abgebaut werden. Dasselbe haben wir bei unseren Versuchen also auch beobachtet, wenn wir den weiteren Abbau mit Erepsin vornahmen. Die Wirkungsweise der Proteosen bei Carrel und Baker ist durch unsere jetzige Kenntnis von der Bedeutung der grösseren Eiweissspaltstücke für den Stoffwechsel der Gewebezellen aufgeklärt.

Wir haben öfter beobachtet, dass die höheren Abbauprodukte kein stärkeres Wachstum der Zellen hervorrufen. Wird aber dialysierter Embryonalextrakt, der selber ohne jeden komplettierenden Einfluss auf dass mangelhafte Medium ist, den *homologen* Produkten zugegeben, so wird das Wachstum dadurch ganz ausserordentlich beschleunigt, ja, es kann sogar ein geringes Wachstum der Zellen bei sonst relativ unwirksamen Abbauprodukten der *heterologen* Eiweisse erfolgen.

Welche Rolle die wirksamen Stoffe des Embryonalextraktes bei dieser Reaktion spielen, wissen wir noch nicht. Ob hierbei Stoffe des Embryonalextraktes die Enzyme an der Oberfläche

der Zellen in irgend einer Weise aktivieren, oder ob Enzyme im Extrakt an der Oberfläche der Zellen verankert werden, lässt sich noch nicht ganz entscheiden. BORGER und PETERS (1933) haben Polypeptidase und Dipeptidase in frischem Embryonalextrakt nachgewiesen. Nachdem wir gesehen haben, dass ein wachstumsförderndes Nukleoproteid, das aus Kalbsembryonalextrakt isoliert wurde (FISCHER 1941), dieselbe Wirkung besass, muss eher vermutet werden, dass die Embryonalextraktwirkung auf einer Aktivierung gewisser Enzymsysteme in der Zelle selbst beruht. In dieser Annahme werden wir noch bestärkt, da LINDERSTRØM-LANG, der freundlichst die entsprechenden Untersuchungen für uns anstellte, weder Dipeptidase- oder Polypeptidaseaktivität noch die Wirkung eines Kathepsins im Nukleoproteid nachweisen konnte.

Die Untersuchungen zeigen deutlich, dass der Artunterschied der bei den genuinen Eiweissen klar zum Ausdruck kommt, auch für die höheren Abbauprodukte, vielleicht zum Teil auch für die niederen, sich geltend macht. Die Wirkung der Abbauprodukte kann in drei Punkten zusammengestellt werden:

1. *Die Wirkung der pepsinabgebauten (P) homologen Eiweisse übertrifft die aller heterologer P;*

2. *Die Wirkung der durch Erepsin (E) abgebauten homologen Eiweisse ist bedeutend geringer als die der entsprechenden homologen P-Produkte;*

3. *Heterologe E-Produkte sind im allgemeinen wirksamer als heterologe P-Produkte.*

Das besagt also, dass höhere homologe Abbauprodukte leichter und besser von den Zellen ausgenutzt werden als niedere homologe Produkte (Gruppe 2). Es ist durchaus wahrscheinlich, dass die Peptide als solche in die Zellen eingeführt werden und direkt zum Aufbau des Cytoplasmaeiweisses dienen (Gruppe 1). Der Bauplan der Polypeptide aus homologem Eiweiss entspricht im wesentlichen dem Bauplan des Proteins der Zellen. Hierfür spricht die Tatsache, dass sie schnell und gut von den Zellen ausgenutzt werden. Im Gegensatz hierzu sind die Peptide aus heterologen Eiweissen entweder garnicht oder doch nur in geringem Grade wirksam, und auch das letztere erst nach einer recht langen Latenzzeit. Um für den Aufbau zu dienen, müssen sie zunächst völlig umgebaut werden. Aus diesem Grunde ist es verständlich, warum die niederen Abbauprodukte der heterologen Eiweisse im allgemeinen wirksamer sind als die entspre-

chenden höheren Produkte (Gruppe 3). Nach dem weitgehenden Abbau werden die einzelnen Säuren für die Synthese besser zugänglich. Ist eine sehr wichtige Aminosäure der Peptidkette abgedeckt, z. B. Cystin, dann bleibt die komplettierende Wirkung völlig aus und die Zellen gehen zu Grunde.

Diese Befunde lassen darauf schliessen, dass das Muster der Peptide entscheidend dafür ist, ob sie von den Zellen verwertet werden können oder nicht. Weiter besagen die Versuche: der enzymatische Apparat der Zellen muss in der Weise eingerichtet sein, dass der Ab- und Aufbau der Eiweiss-Bausteine nach einem ganz bestimmten Plan erfolgt, und dass dieser charakteristisch für jede Tierart, möglicherweise auch für jeden Zelltyp innerhalb desselben Organismus ist. BERGMANN und NIEMANN (1935, 1937 und 1938) haben bekanntlich aus ihren Untersuchungen den Schluss gezogen, dass die einzelnen Aminosäuren in der Peptidkette sich periodisch in bestimmten Abständen wiederholen. Weiter wird von ihnen behauptet, dass es in den Zellen einen Organisator gibt, der den Aufbau der Peptide in dieser Weise steuert. Nehmen wir jetzt an, dass dieser Organisator das Enzym selber ist, so ist es möglich, dass auch der Abbau von Peptiden nur auf solche mit einer bestimmten Konfiguration beschränkt ist.

Diese Gesichtspunkte geben eine natürliche Erklärung für die experimentellen Befunde, die im vorstehenden vorgelegt wurden und stimmen mit den neueren Anschauungen über die Struktur der Peptide gut überein.

Für die Beurteilung der Versuchsergebnisse scheint es uns in diesem Zusammenhang doch wichtig zu überlegen, ob nicht die Unterschiede in den Wirkungen der Abbauprodukte der verschiedenen Eiweisse darauf beruhen könnten, dass das Pepsin unter sonst gleichen Bedingungen nicht in derselben Weise die verschiedenen Eiweisse abbaut. Gegen diese Annahme spricht die Tatsache, dass die verschiedenen Zelltypen nicht in derselben Weise auf dasselbe Produkt reagieren.

Es besteht doch kein Zweifel darüber, dass die höheren, nicht die niederen Abbauprodukte der Eiweisse ein vorzügliches Substrat bilden, um einen Einblick in die Natur der Zelltypen hinsichtlich ihres Eiweissstoffwechsels zu gewähren. So könnte auch ein neuer Weg zur tieferen Erkenntnis der charakteristischen Eigenschaften der Zellen eröffnet werden, wo man sich früher mit vagen morphologischen Merkmalen begnügen musste.

Zusammenfassung.

1. Mischungen von mehr oder weniger hochmolekularen Eiweissabbauprodukten werden von Gewebezellen unter Wachstumserscheinungen ausgenutzt. Die Produkte der *homologen Eiweisse* sind wirksamer als die der *heterologen*.

2. Die mit Erepsin weiter abgebauten *homologen Eiweisse* sind weniger wirksam als die mit Pepsin abgebauten. Umgekehrt verhalten sich die *heterologen* mit Erepsin abgebauten Eiweisse, indem sie im allgemeinen relativ wirksamer als die entsprechenden mit Pepsin abgebauten sind.

3. Der Artunterschied der Eiweisse macht sich auch in der Wirkung der Polypeptide deutlich bemerkbar. Dies steht wahrscheinlich mit Verschiedenheiten im Bauplan der entsprechenden Peptide in Verbindung, d. h. damit, wie sich die einzelnen Aminosäuren in der Peptidkette in bestimmten Abständen periodisch wiederholen.

4. Mit Hilfe der höheren Eiweisspaltprodukte verschiedener Eiweisse lassen sich Gewebezelltypen innerhalb derselben Tierart unterscheiden.

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The Hexosemonophosphoric Acids Formed within the Intestinal Mucosa During Absorption of Fructose, Glucose and Galactose.[1]

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Introduction.

Active transport is caracterised thereby that the cells themselves form the concentration gradients, necessary for their transfer of the substance.

In 1933 LASZT and WILLBRANDT from VERZÁRS institute proposed the theory that the active transport of carbohydrates (hexoses) through cell membranes in the living tissues was made possible by an intermediary phosphorylation of the hexose, the process of phosphorylation taking place in the hexose-absorbing intestinal mucosa. In the same year, 1933, LUNDSGAARD quite independently proposed a similar hypothesis including the glucose absorption of the kidney tubular tissue and the intestinal mucosa. Phloridzin, which was known to prevent the process of reabsorption of glucose in the kidney tubules, now proved to cause a marked and probably specific inhibition of phosphorylation processes. The intestinal absorption of glucose was also found to be sensitive to local phloridzin poisoning; earlier experiments on intestinal glucose absorption had been performed by NAGANO in 1902 with similar results. The various results based on experiments with phloridzin poisoning are still a significant support in favour of the phosphorylation theory.

[1] This publication is abbreviated from a dissertation (chap. V—VIII incl.), not yet published.

LASZT and WILLBRANDT, when they presented their theory, supported it by the observation that when rats were poisoned with monoiodoacetic acid their absorption rate for glucose was reduced to that for xylose. Monoiodoacetic acid has since been proved not to inhibit phosphorylation processes specifically — phosphorylation processes may proceed if the phosphate donors (adenosinetriphosphoric acid) required are present. Phloridzin on the contrary seems to prevent the phosphate transfer from phosphate donor to -acceptor by blocking the phosphate transfer through the adenylic acid system. The oxydo-reductive processes upon which the phosphorylation processes are based may be inhibited unspecifically by iodoacetic acid, relatively high concentrations of which are necessary. Later KLINGHOFFER (1938) has reported that the administration of monoiodoacetic acid, even in small doses, produces widespread tissue damage, the most important, as regards absorption, being hemorrhagic enteritis; the absorption rates of glucose as well of xylose and even chlorides were markedly reduced. Thus the experiments based on phloridzin poisoning must be considered a better support in favour of the phosphorylation theory than the corresponding experiments based on iodoacetic acid poisoning. LAZST (1939) has since reported that the inhibiting effect of iodoacetic acid on glucose absorption could be nullified by a previous injection of sodium chloride or carbonate.

LASZT and SÜLLMANN (1935) demonstrated that the absorption of hexoses and glycerol, but not pentoses, caused an increase in the contents of esterified acid soluble phosphates within the intestinal mucosa. These findings constituted an important support for the phosphorylation theory although the phosphate compounds formed were not identified. LUNDSGAARD (1939) confirmed these findings and found higher phosphate accumulations than had been obtained by LASZT and SÜLLMANN. The higher values obtained in the case of fructose were interpreted as due to slower dephosphorylation, thus accounting for the well-known slower absorption of this sugar. The highest values were obtained in extracts of intestinal mucosas from rats. LUNDSGAARD found similar phosphate accumulations in hexose-absorbing intestines from cats and rabbits; REISER (1940) obtained corresponding values for swine during absorption of glucose.

Only a very few organic phosphorus compounds from the intestinal mucosa have been identified, a fact that is in striking contrast with the size and phosphorylation power of the organ. One of

the compounds so far isolated from the intestinal mucosa is aminoethylphosphoric acid; first of all OUTHOUSE (1937) isolated this compound from malignant tissue. This compound was not, as was at first thought, specific for cancer tissue, for COLOWICK and CORI (1939) have later shown that aminoethylphosphoric acid constitutes about 25 per cent. of the acid-soluble organic phosphates of the small intestine of rabbits. Nothing is known of its function or its source; it is supposed that both aminoethylphosphoric acid and choline phosphoric acid may have a specific yet unknown function in the intestinal tissue, the kidney tissue and tumour tissue. CHARGAFF and KESTON (1940) were unable to show any function of aminoethylphosphoric acid in the mechanism of cephalin formation or transport through the intestinal membrane.

Experimental.

The purpose of these investigations was to isolate and identify the phosphoric compounds formed within the intestinal mucosa during absorption of hexoses.

Only the acid soluble fractions of the intestinal tissues have been analysed.

Methods.

The methods here applied for the examination of intestinal tissue were similar to those applied to fermentation systems by KING and ROBISON (1931), to muscles by CORI and CORI (1931) and to liver tissue by KOSTERLITZ (1939), when he isolated the galactose-1-phosphoric acid from galactose-assimilating livers.

The experiments were carried out on rabbits and rats, fasted 24 hours beforehand. The weight of the animals was about 2 kgms. and 180—200 gms. respectively. Amytal was given during the experimental period.

The hexosephosphoric acids, found within the hexose-absorbing intestinal mucosas, compared to those of the non-absorbing intestinal mucosas might represent the hexosephosphoric acids, accumulated during hexose absorption; therefore a suitable amount of sugar: 5 per cent. glucose, 5 per cent. galactose, or 5 per cent. fructose was administered to the animals orally; the control animals were given a similar volume of 0.9 per cent. sodium chloride.

Whilst the absorption process was still going on, the intestine (jejunum and ileum) was removed and immediately put into ice-cooled 0.9 per cent. sodium chloride solution. The intestinal mucosa was then removed from the muscular tissue on an ice-cooled plate and brought into liquid air, weighed, crushed and extracted with an ice-cooled solution of 10 per cent. trichloroacetic acid. This laborious procedure was necessary in order to avoid the rapid phosphatase breakdown of the

newly formed phosphoric compounds. The extract was filtered and precipitated with pulverized $Ba(OH)_2$ to a pink red phenolphtalein colour. (Special removal of glycogen was unnecessary during the above preparation).

The barium salts precipitated in this manner consisted mainly of inorganic phosphates and presumably of adenosinetriphosphoric acid, hexosediphosphoric acid and 30 per cent. of the hexosemonophosphoric acids originally present. The hexosemonophosphoric acids, precipitated by this procedure, were recovered as described by CORI and CORI.

The phosphoric compounds forming water-soluble barium-salts i. e. hexosemonophosphoric acids, adenylic acid, aminoethylphosphoric acid etc. were precipitated successively by adding 96 per cent. ethylalcohol to the supernatant fluid, the final concentration of alcohol being about 60 per cent; This decrease in water concentration caused the precipitation of the major part of water-soluble barium-phosphates, when the solution was placed in the refrigerator overnight; the precipitate formed was removed by centrifugation. This precipitation was repeated several times. Further addition of alcohol gave a relatively small precipitate, mainly consisting of glycerophosphates.

This procedure by no means allows an exact quantitative nor qualitative determination of the hexosephosphates, but all the same it could be demonstrated that the increase in organic phosphoric compounds, which had accumulated in hexose-absorbing intestines, was reflected in the "hexosemonophosphate fraction". An ideal isolation of some of these compounds could probably be brought about by using a fractionated precipitation of the brucine-salts of these compounds. As the ester-salt amounts yielded by this procedure are relatively small, larger amounts of intestinal tissue should be prepared for this purpose.

Analyses of "hexosephosphate fractions" from intestinal mucosa.

Type of absorption	Rabbits	Rats
NaCl	1	4
glucose	2	5
galactose	—	6
fructose	3	7
glycerol	—	8

Experiments on Rabbits.

1. "Hexosephosphates" from Intestinal Mucosa of Rabbits Absorbing 0.9 per cent. NaCl-Solution; (Preexisting Estors).

Isolation of the phosphate esters:

From 6 rabbits absorbing 0.9 per cent. sodium chloride 141 gms intestinal mucosa were isolated as described above; from the tri-

chloroacetic acid extract of these tissues the water-soluble organic phosphates were precipitated as described; the precipitation procedure was repeated 5 times, a precipitate completely soluble in water and weighing 94 mgs remaining. The results of the analyses are presented in table 1.

2. Determination of "Hexosephosphates" from the Intestinal Mucosa of Rabbits Absorbing Glucose.

From 2 rabbits the intestines were removed, while absorption of glucose was taking place; 48 gms of intestinal mucosa were isolated; from the tissue extracts 22 mgs barium-precipitate were isolated as described above. The results of the analyses are presented in table 1.

Table 1.

Analyses of barium-esterphosphates from intestinal mucosa of rabbits.

Experiment 1, 2 and 3. The values give the composition of 100 mgs barium-esterphosphate. The right column gives the values for iodine — oxidised esterphosphate.

	NaCl-absorbing intestine	Glucose-absorbing intestine	Fructose-absorbing intestine
Barium, mg	25	29	38 (34)
N-contents, mg	(6)	(5)	(5) (3)
Optical rotation			(levo)
Reducing power, HAGEDORN, NORMAN JENSEN, mg glucose	11	48	29 (36)
Aldosecontent, MACLEOD, ROBISON, mg glucose	20	54	17 (0)
Ketose-content, SELIWANOFF, ROE, mg fructose	0.2	0.5	22 (26)
Phosphorus-content:			
Total phosphorus, mg P	3.5	4.6	6.1 (6.9)
P, liberated by hydrolysis, expressed as per cent. of total P. n/HCl, 100° after:			
5 minutes	2	5	42
10 ›	5	9	59
15 ›	8	12	68 (78)
30 ›	11	14	74
60 ›	14	19	75 (84)
180 ›	27	36	79

3. The Demonstration of Fructosephosphoric Acid, Accumulated in the Intestinal Mucosa of Rabbits during Absorption of Fructose.

First of all it was proved that the phosphoric ester formed in the intestinal mucosa during the absorption of fructose was probably a fructose-ester as indicated by a Seliwanoff-reaction: A red colour produced by ketoses (fructose) when heated with resorcinol and HCl. The water-solubility of the barium-salts might show, whether the ester consisted of fructosemonophosphoric acid or of fructosediphosphoric acid; the barium-salts of fructosemonophosphoric acid are soluble in cold and hot water; the barium-salts of fructosediphosphoric acid are only sparingly soluble in hot water, somewhat more soluble in cold water.

Experiment performed in addition to experiment 3 in order to determine the solubility of the barium-salts of the fructose ester:

Analyses of barium-esterphosphate fractions isolated from intestinal mucosa of a fructose absorbing rabbit:

1) Water-soluble barium-esterphosphates (fructosemonophosphoric acid), isolated by one precipitation with alcohol, 60 per cent.:

136 mgs *free* fructose/100 gms intestinal mucosa (free fructose = Seliwanoff-value 2; cfr. table 2)

46 mgs organic P/100 gms intestinal mucosa,

42 mgs N/100 gms intestinal mucosa.

2) Water-soluble barium-esterphosphates precipitated at the first precipitation with $Ba(OH)_2$, but made soluble in water by the recovery process; this fraction consists mainly of fructosemonophosphoric acid:

27 mgs free fructose/100 gms intestinal mucosa,

12 mgs organic P/100 gms intestinal mucosa,

10 mgs N/100 gms intestinal mucosa.

3) Water-insoluble barium-esterphosphates consisting mainly of fructosediphosphoric acid:

5 mgs free fructose/100 gms intestinal mucosa,

15 mgs organic P/100 gms intestinal mucosa,

13 mgs N/100 gms intestinal mucosa.

This experiment showed that the major part of the esters accumulated, if not all, probably consists of fructosemonophosphoric

Table 2.

Composition of barium-salts of various hexosephosphoric acids referred to in text.

Hexosephosphoric acid	Ba, per cent.	P, per cent.	Optical rotation of Ba-salt α 20/D	Reducing power, mg glucose per 100 mgs Ba-salt: Hagedorn, Norman Jensen	Reducing power, mg glucose per 100 mgs Ba-salt: Alkaline Iod	Seliwanoff reaction, mg fructose per 100 mgs Ba-salt
Fructose-1-6-phosphoric acid (Harden, Young ester)	22.5	10.42	+ 1.9°	12	0.7	8
Fructose-1-phosphoric acid	34.5	7.82	− 39.0°	26	0.9	24
Fructose-6-phosphoric acid (Neuberg ester) .	»	7.73	+ 2.3°	34.9	1.6	22
Glucose-1-phosphoric acid (Cori ester)	»	7.8	+ 75.0°	(0)	(0)	(0.5)
Glucose-6-phosphoric acid	»	7.86	+ 29.6°	35.5	45.7	(0.5)

acids. The values obtained from analyses of water-soluble barium-esterphosphates isolated from the intestinal mucosa of 2 rabbits absorbing fructose (table 1) indicated the possibility that the ester accumulated could not be fructose-6-phosphoric acid since the ester proved to be very sensitive to acid hydrolysis. Hydrolysis for 1 hour at 100° C. in n/HCl will liberate about 30 per cent. of the phosphorus present in the fructose-6-phosphoric acid, about 70 per cent. of the phosphorus present in the fructose-1-6-phosphoric acid, and 100 per cent. of the phosphorus present in the fructose-1-phosphoric acid. The Neuberg ester and the fructosediphosphoric acid are well known in biological material, but on the other hand fructose-1-phosphoric ester was, at the time these experiments were performed, only known from yeast preparations, from which the ester has been isolated and identified by MACLEOD and ROBISON (1933) and ROBISON and TANKO (1935). Lately PANY (1942) has isolated this ester together with glucose-1-phosphoric ester from the split products of glycogen formed in liver-pulp incubated for some time at 30° C.

The formation of fructose-1-phosphoric acid in the intestinal mucosa of rabbits absorbing fructose:

In experiment 3 the following more detailed procedure was used in order to determine the nature of the fructose ester accumulated in the intestinal mucosa.

The intestines were removed from 3 rabbits absorbing fructose and extracted with trichloroacetic acid as described above. The phosphoric esters forming water-soluble compounds with barium were precipitated by addition of alcohol at pH about 8, dissolved and re-precipitated 3 times. Some of the phosphate components were removed by precipitation with mercuric acetate from the acidified solution the excess of mercury being precipitated with hydrogen sulphide. The precipitation as barium-salts was then repeated and the precipitate at last dried with alcohol; alcohol-ether; ether and then dried in an exsiccator with P_2O_5 to a white hygroscopic precipitate. The precipitate was weighed and analysed in the following ways:

a) Analysis as in the previous experiments (1 and 2): Table 1. 133 mg barium-esterphosphate were used for this analysis.

b) Another part of the precipitate was oxidized with bromine in order to remove the aldose components, presumably glucose-6-phosphoric acid, present in the precipitate. The procedure should be that used by ROBISON and TANKO in order to prevent the simultaneous oxidation of the ketose-components present; the oxidation products are removed as barium-salts by the precipitation procedure described previously. In the experiment mentioned here however bromine was replaced by iodine under the same conditions as used for the determination of the contents of aldosecomponents in the barium-esterphosphate, MACLEOD and ROBISON. The efficiency of the aldose removal was controlled by the usual aldose-determination made upon the water-soluble fraction, isolated after the oxidation with iodine; Analyses: Table 1.

c) In order to decide whether the phosphoric ester accumulated in the intestinal mucosa consisted of fructose-1-phosphoric acid or fructose-1- + fructose-6-phosphoric acid the following analyses were made:

The acid sensitive fructose-1-phosphoric acid was removed from part of the barium-esterprecipitate by hydrolysis with n/HCl at 100° C. for 60 minutes. By this procedure 4.8 mgs P were liberated together with 26 mgs fructose; the fructose/P proportion being 5.4/1. The theoretical value for fructose-1-phosphoric acid is 6

mgs fructose/mg P. The non-hydrolysed part of the esters was isolated by using the convenient $Ba(OH)_2$, alcohol precipitation. This acid-resistant fraction of the precipitate contained 0.71 mgs P and 0.04 mgs fructose (SELIWANOFF, ROE reaction) while the reducing power (HAGEDORN, NORMANN JENSEN) represented 2.3 mgs glucose.

Thus the content of fructose-6-phosphoric acid in the precipitate proved to be minimal.

d) *Osazoneformation.*

21 mgs of the precipitate originally isolated from the fructose-absorbing intestines were treated with 0.2 cc phenylhydrazine and 0.2 cc concentrated acetic acid at 100° C. for 20 minutes. After cooling the crystals formed were filtered off and recrystallized. The crystals showed great resemblance to those of glucosazone. The melting point was not determined.

The experimental results will be discussed later together with those from the experiments on rats.

Experiments on Rats.

Analyses of water-soluble salts of esterphosphates, isolated from the intestinal mucosa of rats.

Experiment 4. Rats absorbing 0.9 per cent. sodium chloride solution.

6 rats, fasted 24 hours beforehand, had their intestines removed; From the trichloroacetic acid extract of 11.2 gms of intestinal mucosa the esterphosphates forming water-soluble barium-salts were isolated by repeated precipitations with 3 volumes alcohol.

The values for the esterphosphates obtained in the experiments performed on rats are expressed as mgs per 100 mgs dried tissue, as these experiments, contrary to the experiments performed on rabbits, were carried out with special regard to an isolation of the esters as quantitative as possible.

The analytical results are presented in table 3.

Experiment 5. Rats absorbing glucose.

From 6 rats absorbing glucose from a 5 per cent. solution 10.5 gms intestinal mucosa were isolated; the esterphosphates were isolated and analysed as described previously; table 3.

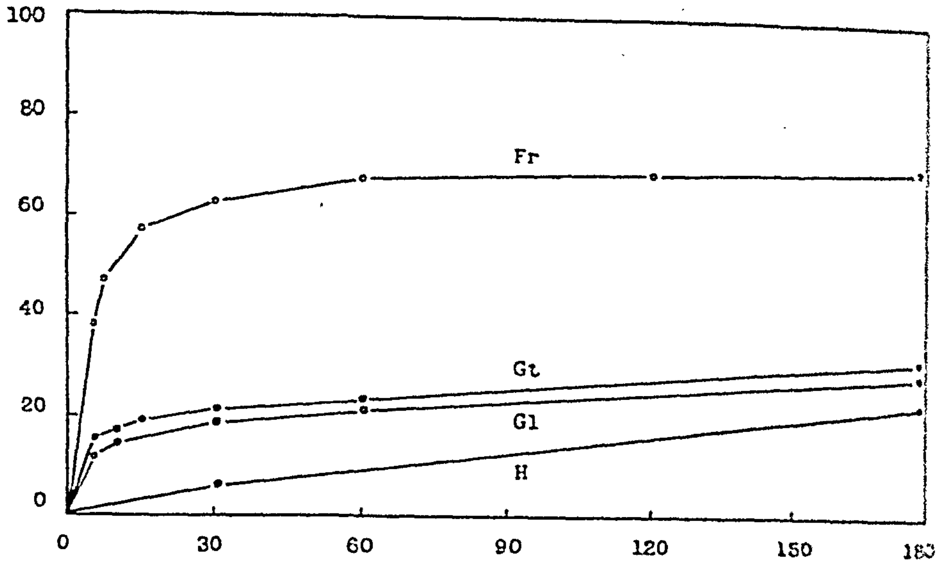


Fig. 1.

Phosphate, liberated by hydrolysis with n/HCl at 100° C. from esterphosphates, isolated from intestinal mucosas of rats: Non-absorbing (H), glucose-absorbing (Gl), galactose-absorbing (Gt) and fructose-absorbing (Fr). Cfr. table 3.
Ordinate: P, liberated; given as per cent. of total P.
Abscissa: Time of hydrolysis; minutes.

Table 3.

The results from analyses performed in experiments 4, 5, 6 and 7.

The values give the contents of 100 mg dried substance. (100 mgs dried substance of intestinal mucosa will correspond to about 500 mg fresh tissue).

Experiment	4	5	6	7
Rats absorbing	sodium chl.	glucose	galactose	fructose
Total phosphorus, mg P	24	26	31	43
Hydrolysis: Fig. 1.				
Reducing Power, HAGEDORN, NORMAN JENSEN, mg glucose	8	100	139	229
Aldose-content MACLEOD, ROBISON, mg glucose . . . :	18	116	202	102
Ketose-content, SELIWANOFF, ROE, mg fructose	0.5	2	2	110
Phloroglucine, HCl-reaction			+ + +	

Experiment 6. Rats absorbing galactose.

From 6 rats absorbing galactose 12.2 gms intestinal mucosa were isolated; the esterphosphates were isolated as described.

The results obtained in this experiment were not sufficient to decide, whether the ester accumulated during the absorption of galactose should be considered a glucose-6-phosphoric ester or a galactose ester, probably a galactose-6-phosphoric ester. A transformation of galactose to glucose in the intestinal mucosa must presumably be based on an initial phosphorylation of the galactose. The only criterion so far available of the formation and accumulation of a galactosephosphoric ester is the intense phloroglucine, HCl-reaction of the barium-esterprecipitate. The phloroglucine-reaction may be carried out in the following way: 0.2 mgs galactose + 1 cc 0.1 per cent. phloroglucine (dissolved in ethyl alcohol) + 5 cc conc. HCl are heated at 100° C. for 3 minutes. The intense red colour developed by this reaction is not stable and rather unspecific. All the same the colour obtained in this experiment was far more intense than that obtained, when the reaction was carried out upon the corresponding precipitate from a non-absorbing intestinal mucosa (the preexisting esters give a weak blue colour (pentose-reaction?); the colour intensity indicated that the major part of the ester mentioned might consist of a galactose ester. A specific absorption by yeast would be of no use, since phosphorylated galactose is presumably assimilated by yeast, KOSTERLITZ.

Experiment 7. Rats absorbing fructose.

From 8 rats absorbing fructose 14.5 gms intestinal mucosa were isolated; the barium salts of the phosphoric esters were isolated in the way described previously. The analytical results are presented in table No. 3.

In a similar experiment 14.0 mgs barium-esterphosphate were isolated from the fructose-absorbing intestinal mucosa of rats. The precipitate, when dissolved in water, showed a typical optical levo-rotation. The total phosphorus content was 5.4 per cent. of the precipitate. After removal of the esters sensitive to acid hydrolysis (n/HCl, 100° C., 60 minutes) a preparation was obtained, which did not contain fructose; the phosphorus content was 0.28 mgs P; reducing power (HAGEDORN, NORMAN JENSEN) expressed as mgs glucose: 1.12. These experiments will be discussed later.

Experiment 8. Rats absorbing glycerol.

From extracts of intestinal mucosa from rats absorbing glycerol and from 6 control animals the ester phosphates were precipitated as barium-salts by addition of 4 volumes ethyl alcohol at pH about 8 and reprecipitated twice. The precipitate was hydrolysed in 2n H_2SO_4 at 100° C. for 6 hours; by this procedure most of the glycerophosphoric acid has been split; the free glycerol was kindly determined by HOLST (8), using a method not yet published. Both glycerol-absorbing intestinal mucosa and the non-absorbing intestinal mucosa contained esterified glycerol probably as α-glycerophosphoric acid, but no significant difference between the contents of the two series of intestines could be proved. The contents of phosphoglyceric acid within the intestinal mucosa estimated by the colour reaction described by RAPAPORT (1937) were negligible.

Conclusion: Experiments Nos. 1, 2, 3, 4, 5, 6, 7 and 8.

I) A certain amount, so far unknown, of the fructose passing through the intestinal membrane during fructose absorption is phosphorylated.

The ester formed within the intestinal walls of rats and rabbits is mainly, if not exclusively, composed of *fructose-1-phosphoric acid*. The existence of this ester within intact animal tissue has not previously been demonstrated.

No fructose-6-phosphoric acid is accumulated within the fructose-absorbing intestinal mucosa. The increased contents of aldose components within the fructose-absorbing intestinal mucosa indicate the simultaneous accumulation to a minor extent of an aldose-ester, probably glucose-6-phosphoric acid.

A minor fraction — not exceeding ten per cent. — of the fructose esters accumulated was precipitated under conditions equal to those of fructose-1-6-phosphoric acid, but it cannot be excluded that this fraction may originate from the fructose-1-phosphoric acid not having been totally removed during the process of recovery.

II) The non-absorbing and the NaCl-absorbing intestinal mucosas of rabbits and rats contained a phosphate-ester fraction that must be considered a glucose-6-phosphoric acid; small amounts of esterified glycerol, probably glycerophosphoric acids, are also present. The proportion N/P of the phosphate compounds forming water soluble barium-salts was found to be 1.8/1; as N/P of the aminoethylphosphoric acid is 0.45/1 only relatively small amounts

of this compound could be present; the presence of some adenylic acid (N/P: 2.2/1) would be a more reasonable guess.

III) The major part of the ester accumulated during absorption of glucose is supposed to consist of glucose-6-phosphoric acid; this conclusion is based upon the values for: a) reducing power, b) aldose-contents, c) resistance to acid hydrolysis, d) the solubility in water of the barium salt of the ester. The increase in hexosemonophosphate fraction from the intestinal mucosa of rats to a minor degree depended on an ester very sensitive to acid hydrolysis; this fraction has not yet been identified; adenosine-triphosphoric acid may be excluded on account of the insolubility of its barium-salt, and therefore there is still the possibility that this ester is glucose-1-phosphoric acid.

IV) During absorption of galactose a phosphate ester will accumulate within the intestinal mucosas of rats; the reducing power towards ferricyanide and iodine, resistance to acid hydrolysis and intense colour reaction with phloroglucine indicate that this ester may be a galactose-6-phosphoric acid. The possibility of a simultaneous accumulation of galactose-1-phosphoric acid is indicated by the occurrence of comparatively small amounts of an ester very sensitive to acid hydrolysis.

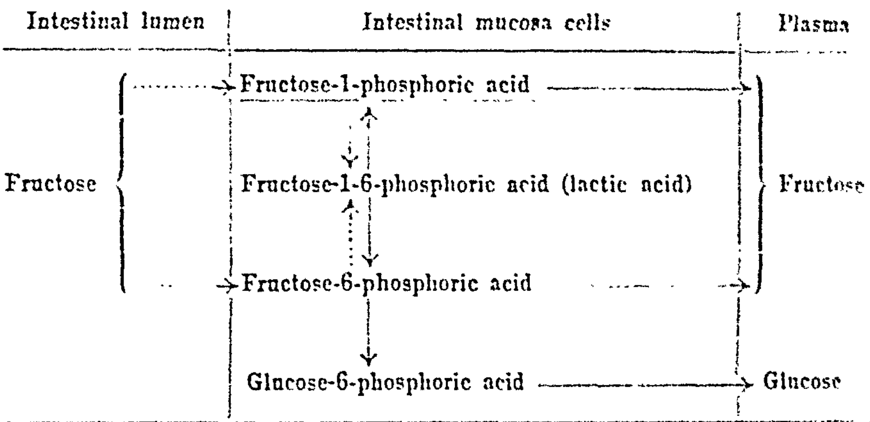
V) The quantity of the esters accumulated may be estimated from the phosphorus analyses of the trichloroacetic acid extracts. Cf. p. 246.

The Significance of the Phosphate Ester Formation in the Intestinal Wall during Absorption.

The accumulations of phosphate ester may be an expression of the fact that the power of the phosphorylation mechanism of the intestinal mucosa is superior to that of the dephosphorylation mechanism. VERZÁR proposed that the relatively low absorption rate of fructose was due to a partial transformation of fructose to glucose in the intestinal wall itself; fructose itself was absorbed by a diffusion process only, while the fructose transformed into glucose, was absorbed selectively at a considerable rate. KALCKAR (1938) confirmed this assumption by experiments showing that *fructose-6-phosphoric acid is selectively dephosphorylated to glucose and phosphoric acid by intact intestinal mucosa phosphatases* comprising LOHMANN's isomerase (phosphohexomutase); this enzyme will transform fructose-6-phosphoric acid to glucose-6-

phosphoric acid without supply of energy. ROBISON and TANKO have shown that only the fructose-6-ester but not the fructose-1-ester, are transformed through this isomerase. GODA (1937) demonstrated that the transformation of fructose into glucose by liver tissue depended upon phosphorylation processes and the interaction of LOHMANN's phosphohexomutase.

The demonstration of the accumulation of fructose-1-phosphoric acid in the intestinal mucosa during absorption of fructose will allow the assumption that the absorption process takes place in accordance with the following diagram:



.... indicate processes depending on supply of energy.
——— indicate processes not depending on supply of energy.

As fructose phosphorylated in position -1- has no possibility of being dephosphorylated selectively i. e. via glucose-ester, the ester accumulated must be the -1-ester. But the fructose-1-ester is also dephosphorylated with great intensity, cf. p. 241.

In connection with the mechanism of absorption proposed above it was investigated, whether the process of transforming fructose into glucose within the intestinal wall took place at any considerable rate:

Experiment 9.

Rabbits that had been fasted 24 hours beforehand were anesthetized with an injection of amytal and given 2 gm fructose/kgm weight in 25 per cent. solution into the intestine. Reducing power (HAGEDORN, NORMAN JENSEN) and fructose (SELIWANOFF, ROE) were determined upon samples of plasma taken simultaneously from the portal vein and carotid artery. The values obtained are given in table 4.

Table 4.

Increase of glucose content of portal blood (plasma) in rabbits during administration of fructose.

The values are expressed in mgs hexose per 100 cc plasma. (P = portal vein; A = carotid artery).

Animal No.	Minutes after administration of fructose	Glucose			Fructose		
		P	A	P—A	P	A	P—A
31	0	207	257	− 50	3	3	0
	5	250	277	− 22	66	29	+ 37
	15	273	292	− 19	77	37	+ 40
32	0	190	242	− 52	2	2	0
	10	226	266	− 40	44	6	+ 38
	25	364	280	+ 84	98	16	+ 82
	40	366	344	+ 22	34	16	+ 18
	55	452	483	− 31	36	39	− 3
33	0	155	180	− 25	1	1	0
	10	254	286	− 14	29	15	+ 14
	40	386	279	− 11	35	16	+ 19
	70	420	444	− 22	28	16	+ 12

Experimental Results.

These experiments show that the transformation of fructose into glucose within the intestine does not take place to any great extent, unless the fructosemia has reached a considerable value; this is seen from the columns indicated (P—A) in table 4, the values of which represent the contents of fructose and glucose, present in the plasma of the portal vein, in excess of those of the arterial blood. Another remarkable fact shown by these experiments was the considerable uptake of glucose (or fructose) by the intestinal tissues; indicated by the negative (P—A) value for glucose; this uptake leads to the assumption that a significant production of lactic acid takes place within these tissues; experiments on this subject are still in progress.

Will Intestinal Phosphatases Be Able to Dephosphorylate the Fructose-1-Phosphoric Acid via the Glucose-6-Phosphoric Acid?

Robison and Tanko (1937) demonstrated that only fructose-6-phosphoric acid was able to be transformed into the aldose ester

under the influence of bone phosphatases containing LOHMANN's phosphohexomutase. The following experiments showed that the same relations towards the fructose-1-ester were found, when intact intestinal mucosa phosphatases were used.

Experimental: Dephosphorylation of fructosephosphoric acid from intestinal mucosa by intestinal phosphatases containing phosphohexomutase.

Phosphatases: Extraction of rabbit intestinal mucosa; 20 gms tissue in 20 cc water; 1 cc extract per sample.

Substrates:

1) fructose-1-phosphoric acid; prepared from fructose-absorbing rabbit intestinal mucosa; fructose-1-ester contents about 60 per cent. 1.01 mgs P per cc.

2) fructose-6-phosphoric acid isolated by acid hydrolysis of fructose-1-6-phosphoric acid followed by precipitation as bariumsalt. 0.94 mgs P per cc.

3) fructose-1-6-phosphoric acid dissolved in water at pH about 8; 0.93 mgs P per cc.

These samples were incubated at pH about 8 and liberation of phosphate and fructose were determined after precipitation with trichloroacetic acid. Table 5.

Experimental Results.

When samples of the same preparations were dephosphorylated with intestinal phosphatases freed from phosphohexokinases as described by ALBERS and ALBERS (1940) — cfr. also KALCKAR — no formation of glucose took place. The results given in table 5 demonstrate that the fructose ester *accumulated* within the intestinal wall is dephosphorylated without any transformation into glucose, and the fructose expressed in per cent. of the fructose present was liberated from the-6-ester and the -1-ester at the same rate. As demonstrated by KALCKAR the transformation of the fructose-6-ester into glucose-6-ester within the intestinal mucosa seems to depend on more complicated dephosphorylating systems within the mucosa tissues.

Table 5.

Dephosphorylation of fructose esters by intestinal mucosa phosphatases containing phosphohexomutase.

Substrate	Minutes incubated	P liberated, mg P	Seliwanoff, mg fructose	Increase in aldose content
0	0	0.08	0	
0	30	0.09	0	+
"Fructose-1-phosphoric acid"	0.5	0.05	1.58	
	1	0.10	1.77	
	30	0.40	1.92	
	60	0.58	1.98	+
Fructose-6-phosphoric acid	0.5	0.04	2.70	
	1	0.10	1.94	
	30	0.22	1.59	
	60	0.46	1.27	+ + +
Fructose-1-6-phosphoric acid	0.5	0.02	1.36	
	1	0.06	1.42	
	30	0.25	0.96	
	60	0.45	0.92	+

Conclusions and the Theory of the Hexose-Phosphorylation as an Integral Part of the Intestinal Absorption Mechanism.

1) *Fructose:* During the intestinal absorption fructose — or a part of the fructose absorbed — is phosphorylated; the ester formed being fructose-1-, fructose-6- or fructose-1-6-phosphoric acid. The formation of fructose-1- and fructose-6-ester is supposed to take place with the same intensity; the energy required must be equal for both cases. The product formed by dephosphorylation is mainly free fructose, although a considerable fraction of the fructose-6-ester present may be transformed into the glucose-6-ester, which is dephosphorylated to glucose and phosphate. This transformation into glucose usually takes place to a minor extent only, and will never exceed 50 per cent. of the fructose absorbed; some of the glucose formed from fructose seems to provide the intestinal mucosa with some of the hexose necessary for supplying energy. The fructose ester accumulated consists of fructose-1-ester, as the only possible breakdown mechanism for this ester is a simple dephosphorylation.

2) *Glucose:* During the absorption of glucose glucosephosphoric esters accumulate in the intestinal mucosa tissue; the ester formed is probably glucose-6-ester accompanied by a relatively small amount of an ester, sensitive to acid hydrolysis and resistent to alkaline hydrolysis (glucose-1-phosphoric acid?). Glucose and galactose are both able to form aldehyde-phosphoric acids. The hypothetic phosphorylating mechanism can be represented as follows:

Intestinal lumen	Intestinal mucosa cells	Plasma
Glucose ⋯⋯⋯⋯	⋯→ Glucose-1-phosphoric acid ——→	Glucose
	↓	
⋯⋯⋯⋯	⋯→ Glucose-6-phosphoric acid ——→	

⋯⋯⋯ processes depending on supply of energy.
——— processes not depending on supply of energy.

A primary phosphorylation of glucose at the aldehyde group has not yet been demonstrated, except when the phosphorylation occurs as an intermediary stage of the phosphorolysis of glycogen. An intermediary formation of glycogen during the absorption of glucose is not probable — the glycogen content of the intestinal tissues is not increased during the absorption process, LUNDSGAARD (1939). The phosphohexomutase present within the intestinal mucosa probably establishes an equilibrium including the glucose-1- and glucose-6-phosphoric acids present. In the experiments carried out by CORI and his collaborators the equilibrium established in vitro was composed of about 19/20 -6-ester and about 1/20 -1-ester. These relations seem to be in some way similar to those of the intestinal mucosa tissues during glucose absorption. It must be supposed that the intestinal phosphatases will dephosphorylate both the -1-ester and the -6-ester.

3) *Galactose:* The galactose-6-phosphoric ester probably formed within the intestinal wall during the absorption of galactose has not yet been proved to exist in animal tissues. It is possible that the composition of the galactose esters formed within the intestinal mucosa is such that an equilibrium composed of galactose-1- and galactose-6-ester similar to that of the glucose esters is established.

KALCKAR in his extensive review (1941) has pointed out that the phosphorylation of an aldehyde group must be looked upon

as a much more expensive process than that of the phosphorylation of an OH-radical as regards the energy required. From this point of view glucose and galactose may be considered as stronger phosphate acceptors than fructose or glycerol, as the energy stored within the esters of the former highly exceeds that stored within the esters of the latter. In this connection it must be mentioned that the absorption of glucose and galactose is more sensitive to phloridzin poisoning of the intestine or to stimulation with thyroxine than that of fructose or pentoses, ALTHAUSEN and Coworkers, LUNDSGAARD (1933), and these experiments.

The Phosphate Renewal of the Esters Formed within the Intestinal Mucosa.

The hexose-phosphates formed within the intestinal mucosa during the absorption of hexoses represent the resultants of both a phosphorylating and dephosphorylating mechanism. If the capacity of the phosphorylating system is superior to that of the dephosphorylating system, some of the esters formed must accumulate. KJERULF-JENSEN and LUNDSGAARD (1941) made an attempt at estimating the intensity of fructosephosphate synthesis within the fructose-absorbing intestine of rats by determining the rate of dephosphorylation of the accumulated ester. In rats, absorbing fructose, phosphorylation was stopped by an intraarterial injection of cyanide; dephosphorylation continued in spite of cyanide poisoning; the intensity of the process was estimated by determining the increase of the inorganic phosphate (mgs per sec.), liberated from the accumulated organic phosphates. The amount of phosphate liberated was found to be equivalent to the amount of fructose absorbed during the same time, which indicated that the theory of intermediary phosphorylation as a hexose absorbing mechanism could be maintained even from a quantitative point of view.

The Phosphate Renewal of the Fructose-1-Phosphoric Acid Accumulated within the Intestinal Mucosa of Fructose-Absorbing Rabbits.

From experiments with radioactive phosphorus HAHN and HEVESY (1940) have shown that the most intense phosphate exchange of the acid soluble fractions of the various organs of rab-

bits is found within the intestinal mucosa tissues. The phosphate exchange observed here was even superior to that of the kidney cortex tissue.

Experimental.

In the following experiment the phosphate exchange of the phosphate ester formed within the intestinal mucosa of rabbits during absorption of fructose was estimated by using a radioactive phosphorus isotope.

A rabbit (weight: 2.1 kgms), anesthetized with amytal, was given by intravenous injection, lasting 4 minutes, 300,000 units ^{32}P as sodium phosphate, dissolved in distilled water; during the injection the animal was absorbing fructose from 50 cc of a 5 per cent. solution, previously introduced. Blood samples were taken during the injection; at the end of the injection the intestines were removed as quickly as possible and immediately cooled. The kidney and the liver were also removed and thrown into liquid air. 35 gms of intestinal mucosa tissue were isolated and extracted with 5 per cent trichloroacetic acid; from the filtrate fractions were isolated: One fraction containing mainly inorganic phosphate, precipitated as ammonium magnesium phosphate; another fraction consisted of "hexosephosphates" i. e. fructose-1-phosphoric acid and to a minor extent of some of the esters preexisting within the intestinal mucosa. This fraction was isolated as barium-esterphosphates. At last the barium esters were hydrolysed for 45 minutes in n/HCl at 100° C.; by this procedure more than $^{9}/_{10}$ of the phosphate present as fructose-phosphate was liberated, while the amount of phosphate liberated from the preexisting esters was negligible. The extracts of kidney cortex tissue and liver tissue were treated in a corresponding manner. The phosphorus contents and activity of all these fractions were determined. The activity (emission of beta-particles) of the radioactive phosphorus was determined in a GEIGER counter arrangement, similar to that described by LEVI (1941). The results are presented in table 6.

The rate of renewal of the ester phosphate is indicated by the incorporation of radioactive phosphate into the ester phosphate fraction from the radioactive inorganic phosphate of the cells, as the radioactive phosphate radicals can only be incorporated into organic molecules in the course of a synthetic process. It is, however, not justifiable to compare the specific activity (activity/mg P) of the ester phosphate extracted from the kidney and the in-

Table 6.

Phosphate exchange of fructose ester, isolated from intestinal mucosa of a rabbit during absorption of fructose.

Intravenous injection of radioactive phosphorus lasting 4 minutes. (The values in the column "average'. indicate that the calculation is based upon the calculated average value of inorganic phosphate after corrections for extracellular P. The values indicated "end" were calculated from the activities at the end of the experiment).

Fraction	Activity/mg P	Activity/mg P in per cent. of Act/mg P for inorg. P	
		"Average"	"End"
Instestinal mucosa:			
Inorg. P	173		
Fructosephospate	115	97	66
Liver tissue:			
Inorganic P	223		
"Esterphosphate"	153	82	69
Kidney cortex tissue:			
Inorganic P	454		
"Esterphosphate"	124	48	27
Plasma, inorganic P.			
After 1 minute	1 840		
» 2 »	1 980		
» 4 »	3 240		

testine, for example, and to conclude from the fact that the ester phosphate extracted from the kidney is much more active than that extracted from the intestine, that the rate of new formation of ester phosphate is correspondingly larger in the kidney. The incorporation of radioactive phosphate radicals into the ester phosphates must be preceded by a diffusion of the labelled inorganic phosphate into the cells of the organ. Thus the rate of formation of labelled ester phosphate is limited by the rate of this diffusion process; in fact, the radioactive inorganic phosphate penetrates very much faster into the kidney cells than into the cells of the intestinal tissue. If a large fraction of the ester phosphate molecules is decomposed and resynthesised more than once during the experiment the inorganic phosphate radicals, which had an activity corresponding to a late stage of the experiment, will be found to a larger extent incorporated in ester phosphate molecules

than those phosphate radicals the activity of which corresponds to an early stage of the experiment. To get proper information on the rate of renewal of an organic compound in an organ, we have to compare the specific activity of the P isolated from the organic compound in question at the end of the experiment with the *average* value of the specific activity of the cellular inorganic P prevailing during the experiment (HAHN and HEVESY).

When the method of calculation, proposed by HAHN and HEVESY (1940) was used, it could be concluded from these results that while absorbing fructose, the rabbit experimented on has synthesized the amount of fructose-monophosphate present in the intestinal mucosa within 4 minutes. This amount of fructose ester was calculated to represent 60—100 mgs of fructose. 3 rabbits of the same weight were found to absorb about 15 mgs fructose per minute under corresponding circumstances. It must be borne in mind that the previous fasting and the amytal injected is able to reduce the absorption rate considerably.

Conclusion: The fructosephosphoric acid synthesis and dephosphorylation in the intestinal mucosa have been found so intense that the theory of an intermediary phosphorylation even of the total amount of fructose absorbed can be maintained.

Summary and Final Conclusion.

1) Some of the fructose, which passes the intestinal membrane of rats and rabbits is phosphorylated. The ester accumulated within the intestinal wall is mainly, if not exclusively, composed of *fructose-1-phosphoric acid.* The existence of this ester within intact animal tissue has not previously been demonstrated.

Fructose-6-phosphoric acid does not accumulate within fructose-absorbing intestines; the simultaneous accumulation of a smaller amount of an aldose ester, probably glucose-6-phosphoric acid, indicates that the fructose-6-ester, when formed is immediately transformed into the aldose ester by the phosphohexomutase present.

A minor fraction of the fructose esters accumulated — not exceeding ten per cent. — was precipitated under the same conditions as fructose-1-6-phosphoric acid, but it cannot be excluded that this fraction may originate from the fructose-1-phosphoric acid not having been totally removed during the precipitations.

2) The non-absorbing and the NaCl-absorbing intestinal muco-

sas of rats and rabbits contain a phosphate ester fraction, which must be considered a glucose-6-phosphoric acid; small amounts of adenylic acid and of esterified glycerol, probably glycerophosphoric acids, are also present.

3) The major part of the ester accumulated during absorption of glucose must be considered a glucose-6-phosphoric acid; evidence is given of the simultaneous occurence of relatively small amounts of glucose-1-phosphoric acid; but this fraction has not yet been identified.

4) During absorption of galactose a phosphate ester will accumulate within the intestinal mucosas of rats; evidence is given *that the* ester *formed is a galactose-6-phosphoric acid.* The possibility of a simultaneous accumulation of galactose-1-phosphoric acid is indicated by the existence of relatively small amounts of an ester, very sensitive to acid hydrolysis.

5) A theory of the phosphorylation processes taking place within the intestinal mucosa during the absorption of hexoses is proposed.

6) The fructose ester, accumulated within the intestinal tissue is not transformed to glucose ester by intestinal phosphatases, in contradistinction to fructose, phosphorylated to fructose-6-ester.

7) *The amount of fructose ester, accumulated within the in*testinal mucosa of rabbits, absorbing fructose, may be calculated to represent 50—100 mgs fructose per animal, weighing 2 kgms.

8) The fructosephosphoric acid synthesis and dephosphorylation of the intestinal mucosa, estimated from experiments with radioactive phosphorus, have proved to be so intense that the theory of an intermediary phosphorylation of even the total amount of fructose absorbed can be maintained.

Final conclusion: It must be pointed out that the demonstration of the hexosephosphate esters formed within the intestinal mucosa from the hexoses being absorbed cannot be looked upon as a final proof of the phosphorylation theory, even though *the latter seems* to have been supported to a great extent. Further investigations on the intensity of the synthesis of the esters compared with the amounts of sugar absorbed may perhaps lead to the conclusion that during its passage through the mucosa cells the hexose absorbed cannot avoid intermediary phosphorylation. As a matter of fact the problem has been limited to the question, whether the hexose, while being absorbed, must necessarily pass through the cells themselves and thereby come into contact with the phos-

phorylating structures of the cells, or whether it may be able to pass the intestinal membrane through the intercellular spaces.

I wish to express my thanks to my chief, Professor E. LUNDSGAARD, for his valuable interest in and kind help with the present work. The preparations of radioactive phosphorus used were presented to me by Professor G. HEVESY, whom I wish to thank for this present and the assistance given to me at the Institute of Theoretical Physics.

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The Phosphate Esters Formed in the Liver Tissue of Rats and Rabbits during Assimilation of Hexoses and Glycerol.[1]

By

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Introduction.

It is now generally accepted that phosphorylation processes form intermediary stages of glycogen or glucose formation from various substances in liver tissue, but the experiments, upon which this assumption is based, have mostly been performed upon isolated tissues. The transformation in vitro of fructose into glucose by liver tissue has for example been described by CORI and SHINE (1936) and GODA (1937). From intact livers of rabbits, absorbing galactose, KOSTERLITZ (1939) has isolated and identified galactose-1-phosphoric acid. LUNDSGAARD (1938) found accumulations of easily hydrolysable, acid soluble phosphates in isolated livers, assimilating fructose.

In order to obtain some information regarding the nature of the phosphate esters formed in the liver tissue during absorption of fructose, galactose, glucose and glycerol the present investigations were performed in coordination with investigations of intestinal mucosa tissues.

Experimental.

The experiments were made on rats and rabbits; all the animals were anesthetized with amytal; the sugars were absorbed from 5 per cent. solutions, which had been introduced into the intestinal lumina. After 15—30 minutes the livers were removed rapidly and thrown into liquid

[1] From a dissertation in press.

air, weighed, crushed and extracted with 10 per cent trichloroacetic acid; 10 cc per gm tissue. The "hexosemonophosphate" fractions of the various extracts were analysed.

Experiments on Rats.

Livers from rats: Non-absorbing (fasted 24 hours beforehand), and livers assimilating: Glucose, galactose and fructose respectively were analysed in order to study the nature of the esters, accumulated.

The four groups of animals each included 5 rats; glycogen was removed from the extracts of the liver tissues with 4 volumes alcohol. Beside glycogen phosphoglyceric acid was precipitated from the acid solution. The bariumsalts of esterphosphates, soluble in water at pH about 8, were precipitated by addition of 4 volumes alcohol; the precipitates formed were centrifuged, dissolved in water and reprecipitated after recovery of esterphosphates from the "insoluble" fraction. The precipitation was repeated 4 times with special regard to a quantitative isolation of the "hexosemonophosphate" fraction; cfr. CORI and CORI (1931) and KOSTERLITZ. The weight of a rat liver was found to be about 3 g liver tissue per 100 g rat.

The experimental results are presented in tables 1 and 2.

Livers from rats analysed in order to demonstrate the occurrence of hexose-1-phosphate esters in liver tissue during assimilation of hexoses.

The "hexosemonophosphates" were isolated as described previously from the livers of 4 groups of rats, each group consisting of 3 animals. One group of rats was fasted 24 hours beforehand, the other groups were absorbing glucose, galactose and fructose respectively. The results are presented in table 3.

Table 1.

Analysis of trichloroacetic acid extracts of liver tissues of rats.

The values indicate mgs P per 100 g of liver tissue.

During absorption of	Glucose	Galactose	Fructose	»Non-assimilating»
P, anorganic	27	23	27	26
P, n/HCl, 5'-P, anorganic . .	11	16	14	10
P, organic	74	73	74	65

Table 2.

Analyses of water-soluble barium-salts of esterposphates, isolated from rat livers.

The values represent the contents of 5 g liver tissue.

Livers, assimilating	Glucose	Galactose	Fructose	»Non-assimilating»
Reducing power, mg glucose .	6.9	7.0	9.3	5.8
Aldose-contents, mg glucose .	9.6	10.0	7.6	7.0
Ketose-contents, mg fructose .	0.2	0.2	2.2	0.2
Phosphorus:				
Total P, mg P	1.2	1.6	1.8	1.2
Hydrolysis: n/HCl, 100° C; per cent of total P.				
5 min.	10	14	18	2
10 »	12	15	26	5
15 »	14	19	30	9
30 »	19	26	34	15
60 »	28	36	38	19
180 »	44	49	51	38

Table 3.

»Hexosemonophosphates» from livers of fasting and hexoseabsorbing rats.

Rats, absorbing	Glucose	Galactose	Fructose	Non-absorbing
Total P; mg per 100 g tissue .	39	29	32	39
P, n/HCl 100° C (per cent. of total P) after 5 min. . . .	20	19	*23*	8
» 30 » . . .	44	30	31	29
P, n/NaOH, 100° C (per cent. of total P) after 3 min. . .	4	6	*12*	4

Experiment 3.

Analyses of liver tissues from rats absorbing glycerol.

The livers were removed from two groups of rats, each group including 3 animals. One group was fasted for 24 hours beforehand, the other had a 3 per cent. solution of glycerol introduced 45 minutes previous to the removal of the livers. The "hexosemonophosphate" fractions, which also contained glycerophosphates were

precipitated by the procedure described previously. Esterified glycerol was partly liberated by acid hydrolysis; n/HCl for 24 hours liberated:

Livers from fasting rats:
12 mgs P per 100 g tissue (50 per cent of total P)
14 mgs glycerol per 100 g tissue.

Livers from rats absorbing glycerol:
14 mgs P per 100 g tissue (50 per cent. of total P).
28 mgs glycerol per 100 g tissue.

The determinations of glycerol were kindly performed by E. Holst by making use of his own method.

The solubility of the barium-salts combined with the considerable resistance towards acid hydrolysis indicate that the glycerol ester fraction is probably mainly composed of glycerophosphoric acid. The ester fraction may be calculated to represent about 100 mgs of glycerol per 100 g liver tissue.

Experiments on Rabbits.

Livers from rabbits: Non-absorbing and assimilating: Glucose or, fructose, analysed in order to demonstrate the nature of the phosphate esters accumulated.

3 groups of rabbits each including 2 animals were treated correspondingly to those of rats in the experiment mentioned previously. The "hexosemonophosphate" fractions of the livers removed were analysed; the analytical results are presented in table 4.

In order to decide whether the esterphosphates, accumulated in the livers of the fructose-absorbing rabbits, contained fructose-6-phosphate ester or not, the esterphosphates were hydrolysed in n/HCl at 100° C for 60 minutes. By this procedure the total amount of phosphate and fructose present as fructose-1-phosphate was liberated and the remaining esters were isolated as barium-salts by repeated precipitation with alcohol at pH about 8; only 30 per cent. of the fructose-6-phosphate present is split by this acid hydrolysis. The esterphosphates resistant to acid hydrolysis were analysed; the results presented below indicate that the contents of ketose i. e. fructose-6-ester must have been minimal:

Phosphorus, mg organic P	0.6
Ketose contents, Seliwanoff, Roe, mg fructose	0.01
Reducing power, Hagedorn, Norman Jensen, mg glucose	2.0

Table 4.

Analyses of esterphosphates, forming watersoluble barium salts, isolated from liver tissues of rabbits.

The values indicate the contents of 100 mgs of bariumesterphosphate. The esterphosphate fraction indicated »fructose)*» has been oxidized with bromine in order to remove the aldosecomponents.

Absorption of	Non. absorbing	Glucose	Fructose	Fructose)*
(Ba-salt, available for the analysis, mgs	30	73	98	30)
Ba, mgs	12	11	15	35
N, mgs	7	7	6	
Optical rotation			(levo)	levo
Red. power, mg glucose; Hagedorn, Jensen	19	21	17	20
Aldose-contents, mg glucose; Macleod, Robison	22	22	16	6
Ketosereaction, mg fructose; Seliwanoff, Roe	0.3	0.5	*4.7*	*9*
Phosphorus:				
Total P, mg P	3.9	4.1	5.4	5.4
P, n/HCl, 100° in per cent of total P,				
at 5 Minutes				14
» 15 »	6	6	*17*	*20*
» 60 »	13	17	25	29
P, n/NaOH, 15°, 15' + J . . .	0	0	0	
P, n/NaOH, 100°, 3' in per cent. of total P			30	22

Similar results were obtained, when barium esterphosphates, soluble in water, were isolated from "non-assimilating" and from fructoseassimilating samples of liver tissue of the same rabbit with special regard to quantitative precipitation. The results are presented in table 5.

While the easily hydrolysable phosphate fraction is increased 175 per cent., the fraction resistant to acid hydrolysis is only increased to 122 per cent. this indicates that the major part of the esters accumulated during assimilation of fructose must be the -1-ester or some other ester, sensitive to acid hydrolysis. The total amount of fructosemonophosphate accumulated may be calculated to about 100 mgs fructosemonophosphoric acid per 100 g liver tissue.

Table 5.

Analyses of esterphosphates from samples of liver tissue from the same rabbit.

The values indicate mgs per 100 g of liver tissue.

Liver assimilating	Fructose	»Non-assimilating»
Seliwanoff, mg fructose	30	2
Phosphorus, mg P:		
P, n/HCl. 100° C, 15 min.	5.1	2.9
P, » » » 60 »	6.1	3.6
P, total	21.2	17.4

Phosphate Renewal of Fructosemonophosphate Accumulated in Liver Tissue during Assimilation of Fructose.

From experiments with radioactive phosphorus, the results of which are presented in table 6 of the preceding publication, it is seen that the phosphate of the phosphate ester accumulated in the liver tissue is renewed with an intensity equivalent to that of the fructosephosphate of the intestinal mucosa of the same rabbit. The phosphate exchange of the ester fraction was found to be more intense than in the experiments performed by Hahn and Hevesy (1940); but the exchange period in the present experiment is only 4 minutes; the latter experimental condition will favour the discovery of an exchange process of great intensity.

Discussion of Experimental Results and Conclusions.

The results of the experiments performed on rats demonstrate that assimilation of glucose, galactose or fructose is followed by an increase in organic phosphorus compounds in the liver tissues of rats. The phosphates accumulated are mainly sensitive to acid hydrolysis, also in the case of fructose assimilation, where the ester formed is very easily hydrolysable. The nature of the phosphate compounds formed and accumulated is to some extent illustrated by the analyses of the barium-salts of the esters, table 2. Analyses of bariumphosphates from livers of rabbits and rats, assimilating fructose (tables 2 and 4) show that the ester in ques-

tion forms a barium-salt, soluble in water at pH 8; it is easily hydrolysed by n/HCl, gives a marked ketose-reaction and reduces potassium-ferri-cyanide. These qualities indicate that a considerable fraction of this ester fraction consists of fructose-1-phosphoric acid. Analyses of the fructose ester fraction resistant to acid hydrolysis and analyses of the bariumesterphosphates, not soluble in water, indicated that the major part of the fructose ester accumulated in these livers is fructose-1-phosphoric acid; the contents of fructose-6-ester may be considered minimal; this agrees with the well known presence of LOHMANN's phosphohexomutase in liver tissues, an enzyme that catalyses the transformation of fructose-6-ester into glucose-6-ester. The possibility exists that an amount of fructose corresponding to that present as fructose-1-ester may be found as aldoseester. The quantity of fructoseester accumulated may be calculated at about 100 mgs of the ester present in 100 g of liver tissue. As during fructose absorption the amounts of fructoseester found in the liver and the intestinal mucosa of the same rabbit seem to be equal with regard to quantity and synthetising intensity, only relatively small amounts of fructose will be able to pass the liver without being phosphorylated.

The phosphate esters accumulated in the liver tissues during absorption of glucose and galactose are probably to some extent aldoseesters i. e. glucose-6-phosphoric acid (or galactose-6-phosphoric acid); this is seen from the fact that aldose contents of the ester fraction from rat livers (but not from rabbit livers) were moderately increased; but the nature of these esters have not yet been verified. Some of the esters accumulated in the livers of both rats and rabbits were found to be very sensitive to acid hydrolysis; as the barium-salts of these esters were soluble in water at pH 8. adenosinetriphosphoric acid may be excluded and the possibility exists that the esters mentioned are glucose-1-ester and galactose-1-ester respectively; these findings seem to be in good agreement with those of KOSTERLITZ, who isolated galactose-1-phosphoric acid from livers of rabbits assimilating galactose. Neither glucose-1-phosphoric acid nor fructose-1-phosphoric acid have yet been isolated from intact liver tissue, but PANY (1942) has recently demonstrated the precence of both esters in extracts from liver pulp of dogs and rabbits incubated for some time at 37°; under these conditions these esters are believed to be formed and accumulated during the phosphorolysis of the glycogen present.

From the increase in easily hydrolysable phosphate during assimilation of galactose (experiment on rats) the amount of galactose-1-ester accumulated in rat livers may be calculated at about 50 mgs ester per 100 g liver tissue. The amount of ester observed by KOSTERLITZ in rabbits was only about 12 mgs ester per 100 g liver tissue. The amounts of esters accumulated in rat livers were larger than those accumulated in rabbit livers, when due regard were taken to the weight of the organs in these animals; this fact may to some extent be a consequence of the smaller rat livers being cooled more rapidly than the larger rabbit livers after the removal of the livers.

During assimilation of glycerol (experiment on rats) the amount of esterfied glycerol (glycerophosphoric acid) preexisting in rat livers of fasting animals was highly increased. The ester fraction could be calculated at about 100 mgs glycerol per 100 g liver tissue. No accumulation of phosphoglyceric acid was observed. KALCKAR (1939) was able to phosphorylate glycerol to α-glycerophosphoric acid in vitro with tissue extracts.

The precipitates of esterphosphates forming water-soluble barium-salts from the hexose-assimilating livers must always be compared with those of the "non-assimilating" livers; the nature of the phosphate esters preexisting in the liver tissues have not yet been examined satisfactorily; some of the barium-salts may consist of more or less broken down nuchleotides: Adenylic acid for example; the contents of nitrogen is relatively high and the blue colour with phloroglucine and HCl indicate that pentoses are present. As a major part of these phosphate esters are resistant to acid hydrolysis and contain aldose-radicals, it seems justifiable to assume the presence of glucose-6-phosphoric acid in liver tissues of fasting animals.

Appendix.

The hexosemonophosphate fraction of kidney tissue and skeleton muscles of rabbits during assimilation of fructose were analysed: No fructosephosphate accumulation was observed in the kidney tissue.

In muscular tissue the assimilation of fructose was accompanied by an increase of the preexisting esters: EMBDEN, ROBISON ester (about 70 per cent. glucose-6-phosphoric acid and 30 per cent. fructose-6-phosphoric acid). No accumulation of fructose-1-phosphoric acid was observed, even when the fructoseemia rose to about

200 mgs fructose per 100 cc plasma by intravenous injection of fructose, but the fructose-6-ester was moderately increased in proportion to the glucose-6-ester.

Summary.

Phosphate esters are accumulated in the intact livers of rats and rabbits during assimilation of hexoses and glycerol, which latter was examined on rats only.

During absorption of fructose the fructose ester accumulated showed qualities similar to those of *fructose-1-phosphoric acid;* no fructose-6-phosphoric acid was accumulated. The amounts of fructosephosphoric acid accumulated in the liver and in the intestinal mucosa of the same rabbit seem to be equal with regard to quantity and synthetising intensity. The amounts of ester may be calculated at about 100 mgs of the ester per 100 g of liver tissue; this amount was rebuilt almost completely within 4 minutes as shown in an experiment with radioactive phosphorus.

The phosphate esters accumulated in livers of rats during absorption of glucose and galactose are probably to some extent aldose-esters i. e. glucose-6-phosphoric acid (and galactose-6-phosphoric acid?). Some of the esters accumulated during absorption of glucose and galactose in the livers of rats and rabbits were very sensitive to acid hydrolysis and relatively resistant to hydrolysis with alkali; the possibility exists that these esters are glucose-1- and galactose-1-esters respectively.

During absorption of glycerol an amount of glycerol representing about 100 mgs glycerol per 100 g liver tissue of rats was esterified; as phosphoglyceric acid and triosephosphoric acid could be excluded, the ester in believed to be (α- ?)-glycerophosphoric acid.

No accumulation of fructose-1-phosphoric acid was observed in the kidneys or muscular tissue of rabbits during assimilation of fructose, but the contents of fructose-6-ester of the preexisting EMBDEN-ester was somewhat increased.

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A Reticulocyte Ripening Principle.

By

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(Received 23 May 1942.)

When stained supravitally with basic dyes some red blood corpuscles show a "reticulated substance" consisting of fine or coarse granules connected with each other by a fine filament. Such erythrocytes are called *reticulocytes*. In the blood of normal animals between 0 and 30 per mille of the total number of erythrocytes are found to be reticulocytes varying with the species and the age of the animal examined. Now the reticulocytes generally are accepted as young red blood cells. Whenever the blood formation in the bone marrow is more active the number of reticulocytes in the blood increases. This phenomenon has caused the importance of reticulocytes in the clinic where extensive studies have been carried out on this subject.

Pure physiological investigations on reticulocytes are rather scanty and our knowledge here limited.

The present paper reports a study of the ripening process of the reticulocytes into mature non-reticulated erythrocytes.

When blood is stored the reticulated erythrocytes gradually disappear. This was shown for the first time by PEPPER (1922) who incubated human or rabbits' blood stabilised with citrate at body temperature while blood stored at 4° showed no alteration in this respect. The same was seen by SEYFAHRT (1927). HEATH and DALAND (1930) found that reticulocytes in vitro at 37° or in the pleural cavity of the rabbit could after a few days no longer be identified by supravital staining, decreasing at a regular rate from the first day. The rate was similar for reticulocytes from rabbits which had been bled or made anemic with phenylhydrazin, and from patients with hemolytic jaundice or pernicious anemia. The

rate was slower at lower temperatures and reticulocytes persisted in blood kept in the ice box for six months. KRAFTA (1931) concluded that a temperature coefficient for the loss of specific staining qualities in animals after death is indicated and is of the order of that for chemical reactions.

The mechanism of this disappearance of the reticulated cells has been the subject of several investigations though merely from a morphological point of view.

The amount and the appearence of the reticulum in the cells varies. CESARIS-DEMEL (1907) found different types of reticulocytes according to the amount of stainable substances in the cells. Investigations of SEYFAHRT (1927), SEYFAHRT and JÜRGENS (1928), MOLDAWSKY (1928), GAWRILOW (1929), RIDDLE (1930), HEATH and DALAND (1930), ROSIN and BIBERGEIL (1904) showed that the types containing much of the substance which aggregates in the form of a reticulum are to be regarded as younger cells while the cells containing lesser amounts are to be regarded as older stages of the reticulocytes. The comparative age of the reticulocytes can be estimated from the stained film, the heavily clumped and wreathed forms being the most immature and the sparely reticular and punctuate forms, the older cells. RIDDLE (1930) estimated from investigations on patients with pernicious anemia that reticulocytes with very little reticulum material disappear within 24 hours, but that younger reticulocytes differentiate into mature erythrocytes in from two to four days, depending on the intensity of the stimulus to regenerating.

Experiments with blood incubated in vitro show that first the older forms disappear and later the younger forms (HEILMEYER and WESTHÄUSER (1932)). Both PEPPER (1922) and later on HEATH and DALAND (1930) found that the reticulocytes were not replaced by structureless adult erythrocytes but by cells containing stainable granules. The cells mentioned by PEPPER (1922) and HEATH and DALAND (1930) must, however, be regarded as the oldest stage of the reticulocytes; and the results of the countings by HEILMEYER and WESTHÄUSER (1932) show that even this type of reticulocytes disappears when the blood is incubated long enough.

Furthermore it is shown that all erythroblasts containing hemoglobin are heavily loaded with reticulum. From this fact and from the investigations mentioned it must be concluded that the reticulocytes are unripe red blood cells which, from types with clumped

reticulum through types with decreased amounts ripen into mature erythrocytes.

Several authors have estimated the duration of the reticulocytar stage and find the following figures:

PEPPER (1922)	72	hours
DENECKE (1923)	72	»
RIDDLE (1930)	24—72	»
SEYFAHRT (1927)	24	»
HEILMEYER and WESTHÄUSER (1932)	48	»
GORDON and KLEINBERG (1938)	96—144	»
HEATH and DALAND (1930)	72—96	»

Recapitulating our knowledge of the ripening process of the reticulocytes it is stated 1) that the reticulocytes ripen through more mature types into normal "adult" erythrocytes, 2) that the ripening process even goes on in vitro, and 3) that it is accelerated with increasing temperature. In the present and in following papers it will be shown that the ripening process is not spontaneous but induced by substances found in the blood plasma and in some organs and tissues in the organism.

Technique.

The reticulocytes in rabbits' blood were used. Only males weighing from 2,000—2,500 grams were used; they were kept singly and fed with turnips and cabbage leaves. When fed with hay additional water must be given. In order to obtain a reasonable accuracy in counting the reticulocytes the animals were brought into a chronic anemic state through bleeding which is accompanied by a considerable reticulocytosis. This was done either by heart puncture or by bleeding from the ear into the SJÖVALL apparatus (1936). Daily 30—50 ml. of the blood was taken.

After a week's treatment the animals get a hemoglobin percentage of 40—50 and a red blood cell count of 2—3 mill. per c.mm. with 20—30 per cent reticulocytes. This state can be maintained during a month or more before the animals die.

In a ripening experiment 40 ml. blood was taken in 10 ml. 3.8 per cent solution of sodiumcitrate. After scrupulous mixing, portions of 2.50 ml. were put into 10 ml. centrifuge tubes each containing 2—3 glass beads.

Before each test the blood must be mixed thoroughly for at least 2 minutes, without shaking, in order to secure the same percentage of reticulocytes in each tube as the reticulocytes sediment more slowly than the mature erythrocytes. The tubes are then centrifuged for 5 minutes at 3—4,000 revolutions per min. The plasma is sucked off and

the blood cells washed with 5 ml. 0.9 per cent sodium chloride which after a second centrifugation is discarded.

The solution to be examined are then added to the washed erythrocytes, mixed at least for 1 minute, and placed in four small test tubes with 2—3 glass beads in each. The test tubes are closed with rubber stoppers and given the numbers I—IV. The reticulocyte percentage in tube I is determined at once while the others are placed in a thermostat and rotated vertically at the rate of 30—35 revolutions per minute. Tube II is examined after 2 hours, tube III after 4, and tube IV after 6 hours incubation.

The counting of the reticulocytes was performed in the following way: In a small test tube are placed 2—3 glass beads and 0.5 ml. of a solution of 0.15 g brilliant cresyl blue in 100 ml. 0.9 per cent NaCl. After mixing the blood to be tested for 1 minute (stopwatch) two or three drops of the blood are added to the solution of the dye, mixed, and kept for 20 minutes at room temperature. The content of the tubes is then mixed for exactly 1 minute and a drop placed on a slide and covered with a cover glass. The drop must be large enough to fill the entire space between slide and coverglass.

We counted 2 × 500 red blood cells. 75 reticulocytes per 500 cells gave a mean error of ± 2.7 reticulocytes corresponding to 15.0 per cent reticulocytes ± 0.54 and 10 reticulocytes per 500 cells gave a mean error of ± 2.2 corresponding to 2.0 per cent reticulocytes ± 0.44 i. e. the error increases with decreasing percentage of the reticulocytes.

Results.

Table 1 shows the result of a typical experiment.

Table 1.

Fall in the reticulocyte number by incubation at 40° in saline, plasma, and saline with liver extract. No haemolysis in any tube.

Glass No.	Red blood cells suspened in 0.9 per cent NaCl		Red blood cells suspended in rabbits' plasma		Red blood cells suspended in 0.9 per cent NaCl with 1 per cent concentrated liver extract (Hepsol fort. MCO)	
	Per cent reticulocytes	Millions of erythrocytes per c.mm.	Per cent reticulocytes	Millions of erythrocytes per c.mm.	Per cent reticulocytes	Millions of erythrocytes per c.mm.
I Before incubation . .	11.4	3.02	11.0	3.00	10.5	3.06
II 2 hours at 40°	10.8	2.96	9.9	3.12	9.2	3.08
III 4 hours at 40°	10.2	2.90	8.8	2.92	8.1	2.91
IV 6 hours at 40°	9.5	2.94	7.9	2.83	6.9	2.86

It is seen that the "spontaneous" decline in reticulocytes when incubated in physiological salt solution is only slight and that it can be accelerated considerably when the incubation is performed in plasma or when a small amount of liver extract is added to the saline solution. Thus the latter mediums must contain principles activating the disappearance of the reticulocytes.

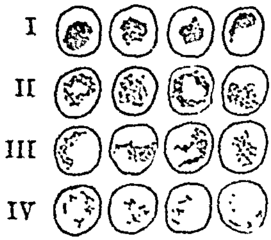

Fig. 1. Differentiation of the reticulocytes.

Group I. Heavily clumped forms.
The reticulum is very intense and lies in the centre of the cell, often it has the form of a ring.

Group II. Wreathed forms.
The reticulum lies as a wide meshed net covering most of the cell.

Group III. Sparsely reticular forms.
The structure of the net begins to disintegrate but the greater part of it is still preserved.

Group IV. Punctuate forms.
The supravital stainable substance is mainly seen in the form of granules of which only a few are connected with each other by a filament.

It can be clearly demonstrated that this disappearance is due to a ripening process. According to the age of the reticulocytes these cells appear in different types. I have used the following differentiation described by TRACHTENBERG (1932) and applied to reticulocytes incubated in vitro by HEILMEYER and WESTHÄUSER (1932) (Fig. 1).

During an incubation experiment the different groups were altered as shown in Table 2.

It is seen that the disappearance of the reticulocytes occurs in the younger groups and the younger the group, the more marked the result. The number of reticulocytes in group IV is mainly unchanged and this is in accordance with the abovementioned findings by PEPPER (1922) and by HEATH and DALAND (1930). This means that the ripening of group IV into mature red blood corpuscles progresses at the same rate as the ripening of the other groups into type four.

Table 2.

Per mille reticulocytes of the different groups during incubation.

Glass No.	Incubated in 0.9 per cent NaCl					Incubated in rabbits' plasma					Incubated in 0.9 per cent NaCl + 1 per cent liver extract				
	I	II	III	IV	total	I	II	III	IV	total	I	II	III	IV	total
I Before incubation	36	44	57	61	198	37	53	53	61	204	37	47	52	58	194
II 2 hours incubation	31	42	58	62	193	26	49	53	60	188	22	45	50	50	167
III 4 hours incubation	26	45	54	61	186	24	37	44	64	169	13	30	49	57	149
IV 6 hours incubation	23	43	53	59	178	17	36	38	66	157	7	21	32	59	119

The ripening process of the reticulocytes follows the monomolecular equation:

$$k = \frac{1}{t} \log. \frac{a}{a - x}$$

where t is the time of incubation, here in hours, a the number of reticulocytes at the beginning of the experiment, and x the number that disappeared during t. (Table 3.)

Table 3.

The decrease of reticulocytes follows the monomolecular equation.

Glass No.	Incubated in 0.9 per cent NaCl		0.9 per cent NaCl + 1/2 per cent liver extract		0.9 per cent NaCl + 1 per cent liver extract		0.9 per cent NaCl + 2 per cent liver extract	
	Per mille reticulocytes	$\frac{1}{t} \log \frac{a}{a-x}$	Per mille reticulocytes	$\frac{1}{t} \log \frac{a}{a-x}$	Per mille reticulocytes	$\frac{1}{t} \log \frac{a}{a-x}$	Per mille reticulocytes	$\frac{1}{t} \log \frac{a}{a-x}$
I Before incubination	136		130		140		134	
II 2 hours incubination	128	0.0144	113	0.0303	112	0.0485	90	0.0870
III 4 hours incubination	124	0.0103	98	0.0312	86	0.0519	66	0.0775
IV 6 hours incubination	116	0.0118	83	0.0323	69	0.0516	47	0.0769
Average of constants	0.0122		0.0315		0.0507		0.0802	

It is seen from Table 3 and yet more from the following Tables 4 and 5 that the monomolecular constant k depends on the concentration of ripening substances in the suspending medium. If the monomolecular constant from the spontaneous ripening pro-

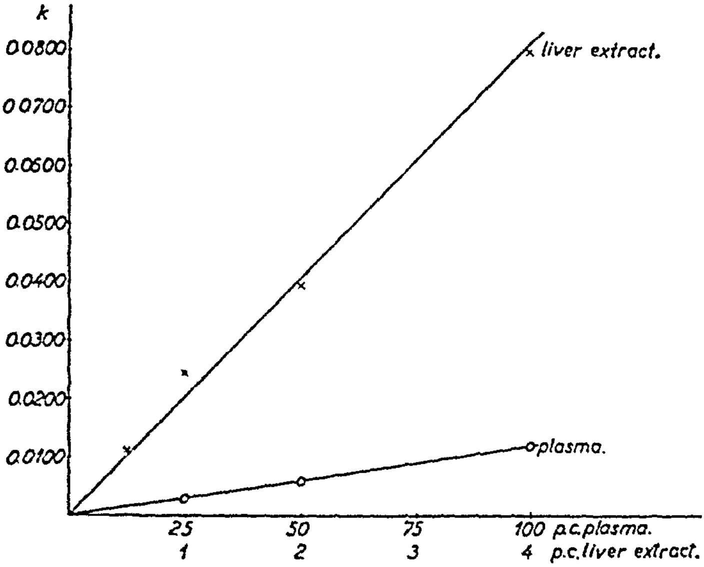

Fig. 2. Dependences of constants on concentration of ripening substances.

cess in saline, k_s, is subtracted from k_f, the monomolecular constant found when some ripening medium is added, the real ripening constant, k, will be found. This constant is proportional to the concentration of the unknown ripening principles contained in the solution. Table 4 shows this with liver extract and Table 5 the same with diluted plasma (see also Fig. 2).

Table 4.

Dependence of constants on concentration of ripening substances (liver extract).

	0.9 per cent NaCl	0.9 per cent NaCl with addition of liver extract			
		0.5 per cent	1.0 per cent	2.0 per cent	4.0 per cent
k_s	0.0136	—	—	—	—
k_f	—	0.0248	0.0381	0.0534	0.0943
$k_f \div k_s = k$ . . .	0	0.0109	0.0245	0.0398	0.0807

Table 5.

Dependence of constants on concentration of ripening substances (plasma).

	0.9 per cent NaCl	0.9 per cent NaCl diluted with		
		25 per cent plasma	50 per cent plasma	100 per cent plasma
k_s	0.0116	—	—	—
k_f	—	0.0145	0.0175	0.0235
$k_f \div k_s = k$	0	0.0029	0.0059	0.0119

The constant k does not depend on the absolute amount of ripening substances used, only on the concentration as shown in Table 6; the same quantity of blood corpuscles are suspended in 1, 2, 4, or 10 ml. The constants k_s and k_f and with them k remain unaltered.

Table 6.

Independence of monomolecular constants of the amount of suspending liquid.

	0.5 c.c. blood corpuscles suspended in			
	1 c.c.	2 c.c.	4 c.c.	10 c.c.
0.9 per cent NaCl k_s	0.0115	0.0115	0.0117	0.0114
0.9 per cent with 1 per cent liver extract. . . . k_f	0.0271	0.0269	0.0264	0.0266
Plasma . . . . . k_f	0.0234	0.0229	0.0225	0.0230

Table 7.

Dependence of k_s, k_f, and k on the temperature of incubation.

Temperature of incubation:		4°	10°	20°	30°	37°	40°
Incubated in 0.9 per cent NaCl	k_s	0.0000	0.0000	0.0016	0.0040	0.0074	0.0095
Incubated in plasma (rabbit)	k_f	0.0000	0.0016	0.0061	0.0149	0.0244	0.0301
	k	0.0000	0.0016	0.0045	0.0109	0.0170	0.0226
Incubated in 0.9 per cent NaCl + 1 per cent liver extract .	k_f	0.0000	0.0022	0.0071	0.0181	0.0286	0.0361
	k	0.0000	0.0022	0.0055	0.0141	0.0212	0.0266

As could be expected the constants depend very much on the temperature. Table 7 shows the results. Temperatures above 40° cause hemolysis. When the constants found at the temperatures investigated are put in relation to the constants obtained at 40° it can be shown that the temperature coefficient is the same regardless of whether the maturation is induced spontaneously, by liver extract, or by plasma (Table 8).

Table 8.

Relative variance of k with the temperature of incubation.

Temperature of incubation	4°	10°	20°	30°	37°	40°
Incubated in 0.9 per cent NaCl. Variance of k_s k_s 40° = 100	0	0	16	42	80	100
Incubated in plasma. Variance of k k 40 = 100 .	0	7	20	48	75	100
Incubated in 0.9 per cent NaCl with 1 per cent liver extract. Variance of k 40° = 100	0	8	21	53	80	100

Table 9.

Constants found on different days working with blood corpuscles from rabbit 145 using 1 per cent liver extract 0—66 as a ripening substance.

Date	Per mille reticulocytes	k_s	k_f from 1 per cent liver extract NO. 0—66	k
May 16, 1941 . . .	62	0.0082	0.0309	0.0227
May 27, 1941 . . .	67	0.0091	0.0308	0.0217
June 2, 1941 . . .	82	0.0082	0.0301	0.0216
June 4, 1941 . . .	91	0.0093	0.0317	0.0224
June 6, 1941 . . .	102	0.0098	0.0304	0.0206
June 9, 1941 . . .	104	0.0093	0.0307	0.0214
June 13, 1941 . . .	112	0.0093	0.0320	0.0227
June 19, 1941 . . .	134	0.0089	0.0316	0.0227
June 23, 1941 . . .	156	0.0091	0.0314	0.0223
June 25, 1941 . . .	188	0.0097	0.0323	0.0226

The experiments given in Tables 6 and 7 were not made on one and the same day but they were performed with blood corpuscles from the same rabbit. It is evident that the constants found from day to day with blood cells from the same animal and with the same concentration of ripening substances from the same batch, are the same regardless of the original number of reticulocytes in the blood.

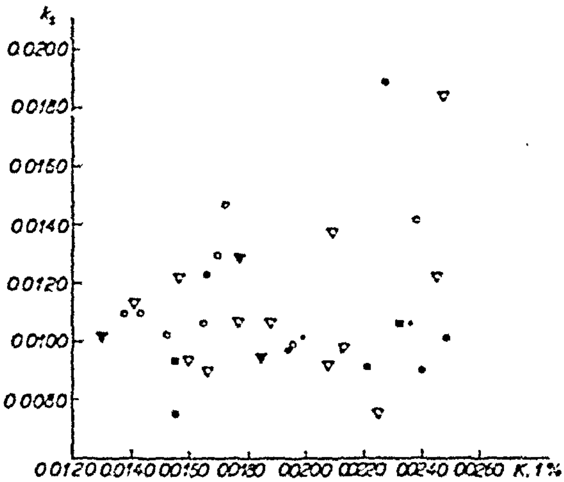

Fig. 3. Relation between spontaneous and induced maturation.

▽ single determination
○ average of 2 determinations
▼ » » 3 »
● » » 4 »
■ » » 5 »
Liver extract 0—66.

An example is given in Table 9 and the same is found in several other similar experiments.

When blood from different rabbits are used as objects k_1, k_{II} and k are, however, found to vary from rabbit to rabbit. The lowest k_1 found among the first 31 rabbits was 0.0075 and the highest found 0.0189. When 1 per cent liver extract 0—66 is used k varies among the same 31 rabbits between 0.0130 and 0.0247.

Fig. 3 shows the values for k_1 and k on the 31 first rabbits examined. Two animals show relatively high spontaneous ripening velocity and are above average of the k values found. Apart from this no relation between the ability to spontaneous ripening and the ability to induce ripening is seen.

Table 10.

The testing of some liver extracts by the use of a standard.

Date	Rabbit No.	Per mille reticulocytes	0.9 per cent NaCl k_s	1 per cent liver extract 0—66 standard		Liver extract to be tested	1 per cent liver extract to be tested		$\frac{k \text{ liver extract}}{k \text{ standard}}$
				k_t	k		k_t	k	
July 24' . .	153	159	0.0112	0.0356	0.0244	»Hepsol»	0.0172	0.0060	0.246
July 25' . .	68	68	0.0083	0.0331	0.0248	»	0.0151	0.0068	0.274
Dec. 1' . .	7	193	0.0094	0.0274	0.0180	0—96	0.0258	0.0164	0.914
Dec. 4' . .	21	167	0.0118	0.0321	0.0203	»	0.0302	0.0184	0.914
Nov. 18' . .	19	203	0.0122	0.0279	0.0157	1—119	0.0405	0.0287	1.83
Nov. 19' . .	7	233	0.0099	0.0283	0.0184	»	0.0416	0.0317	1.72
Nov. 20' . .	21	147	0.0102	0.0261	0.0159	»	0.0391	0.0289	1.82
Aug. 7' . .	118	173	0.0074	0.0292	0.0218	0—85	0.0307	0.0233	1.07
Sept. 3' . .	190	106	0.0090	0.0274	0.0184	»	0.0285	0.0195	1.06

As k varies $\pm$ 25 per cent from the mean with the rabbit which gives the reticulocytes applied in the experiment we have found it necessary to use a *standard* when determining the concentration of ripening substances in solutions to be tested.

When this is done, the results obtained with the different rabbits can be compared. As a *unit* we have defined the concentration of ripening substances in ox plasma for reasons I shall give in a following paper. Our standard, 1 per cent liver extract-Hepsol fortior — batch 0—66 contains approximately 0.97 unit.

Finally in Table 10 I shall give an example of the testing of different batches of liver extract showing that when the standard is used the results can be reproduced with fair accuracy.

Summary.

When reticulocytes in vitro are suspended in physiological salt water and incubated at 40° the reticulocytes disappear only very slowly.

The rate of disappearance can be accelerated considerably by adding liver extract to the salt solution or when the reticulocytes are suspended in plasma.

The youngest and most unripe types disappear first. The disappearance thus must be due to a maturation of the reticulocytes into "adult" erythrocytes and this process must be accelerated by some unknown ripening substances found in plasma and liver extract.

The ripening process follows the monomolecular equation.

The monomolecular constant is proportional to the concentration of ripening substances in the suspending solution.

The monomolecular constant increases with increasing temperature.

When reticulocytes from the same animal are used, the monomolecular constant found at different times is the same if the same concentration of ripening substances is used in the experiments.

The monomolecular constant can vary $\pm$ 25 per cent when reticulocytes from different animals are used. The proportion between two tested solutions is, however, always the same, independent of the animals used.

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From the Biol. Laboratories, Medicinalco, Copenhagen S.

On the Chemical Nature of the Reticulocyte Ripening Principles in Liver.

By

ERIK JACOBSEN and CLAUS MUNK PLUM.

(Received 23 May 1942.)

In an earlier paper PLUM (1942) has proved the existence of certain principles in blood plasma and liver extracts able to accelerate the ripening of reticulocytes in vitro. In the present paper we describe some chemical properties of these principles.

As the ripening substances are found abundantly in liver extracts for parenteral use we have used this in our attempts to isolate the active factor or factors.

Very early it was shown that the reticulocyte ripening principle was thermolabile as liver extracts lose their activity after heating 5 minutes on a boiling water bath. Thus it cannot be identical with the principle active against pernicious anemia which in liver is heat stable.

Our experiments were carried out with a liver extract prepared according to KYER (1935).

Preliminary experiments with saturation with ammonium sulphate gave no complete precipitation of the active principle and precipitations with Reinicke salt gave only poor yields and little concentration of the active principle. Very early we found that the liver extract lost its reticulocyte ripening power after being treated with floridin practically without loss of the remaining dry substances. It was, however, difficult to elute the principles from the floridin. Diluted hydrochloric acid and diluted ammonia proved ineffective in this respect. We found that 70 per cent alcohol and liquid phenol was able to elute the reticulocyte

ripening principle. The phenol, however, gave a more concentrated yield. The concentration was several hundred times as it was found active in concentrations of less than 1 : 100,000 of dry matter but the yield was rather small. As the loss was considerable we imagined that the effect of the principle was due to several factors. Hence we tried to add liver extract, made inactive by shaking with floridin, to the floridin eluate and really found a considerable rise in the reticulocyte ripening power of the latter. Thus the solution of the non-absorbable substances which per se is inactive, must contain some factor or factors able to increase the activity of the reticulocyte ripening factor being absorbed by floridin.

Chemical Nature of Activating Factors in the Non-Absorbable Part.

The activating factor is thermostabile and stands boiling for several hours. We tried to precipitate this factor with Reinicke salt, phosphotungstic acid, silver-, mercury-, copper-, and lead-salts without satisfactory results. We found, however, that the activating factors could be extracted with n-butanol from which after concentration it could be precipitated with ethyl ether. This procedure gave a concentration of five to ten times. After the precipitate was dissolved in water and ethanol added, 1—2 per cent of the total dry substance was precipitated. This precipitate was very active and was identified as *l-tyrosine.* Experiments showed that synthetic l-tyrosine had the same activating effect as the tyrosine isolated from the liver extract. Furthermore, estimations of tyrosine according to GREENBERG (1929) proved that the total activating power of the butanol extract must be due to the tyrosine found in this fraction. After the precipitation of the tyrosine the fraction showed practically no activating effect. Comparing the effect of the liver extracts absorbed by floridin with water solutions containing the same concentration of tyrosine, we found that the pure tyrosine solutions had a greater activating effect than could be expected from the tyrosine analyses of the liver extract. This can be due to the fact that the tyrosine analyses applied are not specific as they react on phenol groups. Another explanation is that the liver extracts contain some counteracting substances. We have actually found inhibiting substances in some of the fractions prepared.

Experimental.

The method of preparation is summarised in table 1.

Table 1.

Scheme of preparation.

Liver extract 0—85 dry matter 72 g in 500 ml.

absorbed by floridin eluted with phenol
Fraction H
dry matter 60—65 mg

not absorbed by floridin
dry matter 72 g
Fraction D

extracted with butanol, precipitated with ether
Fraction DK
dry matter
5—8 g

Fraction DJ
dry matter
65 g

dissolved in water precipitated with alcohol
Tyrosine 0.2 g

500 ml liver extract 0—85 with 14.4 per cent dry matter and each ml corresponding to 190 g fresh liver is shaken for one hour with 10 g floridin and sucked off. The floridin is washed with 100 ml distilled water and shaken twice with 100 ml 70 per cent alcohol sucked off and dried. Then the floridin is eluted for one hour with 40 ml liquid phenol at 40°—45°; this is repeated three times in all. The combined phenol phases, total 125—130 ml, are dissolved in 3 litres distilled water which is shaken four times with each 1.5 litre ethyl ether in a separatory funnel. The water is then evaporated under reduced pressure to 250 c. c. This fraction is called *fraction H*. Its content of dry matter is 25 mg per 100 c. c.

The filtrate from the absorption is called *fraction D* which contains 14.4 per cent dry matter.

100 ml of fraction D is shaken eight times with each 100 ml normal butanol. From the fourth or fifth extraction the water phase is very concentrated and must be diluted to 50 ml before each further extraction. The collected butanol extractions are cleared by centrifugation and evaporated under reduced pressure to a total volume of 100 ml. When the water in the butanol is driven off a white substance precipitates as the extracted matter is less soluble in pure than in watery butanol. To the residue and precipitate is added 300 ml ether. The brownish precipitate is centrifugated off, washed three times with ether and dried in an exsiccator over sulphuric acid in vacuo. As it is very hydroscopic it must be stored under similar conditions. Yield of this *fraction*

DK is 1.2—1.5 g per 100 ml. D extracted. 10 g DK are dissolved in 40 ml distilled water and 40 ml alcohol added; the white precipitate is filtrated off, washed with little ice water and alcohol, and dried. Yield 0.34 g. Tyrosine analyses of this substance according to GREENBERG (1929) gave 98 per cent tyrosine.

Elementary Analyses.

C	found	59.3 per cent,	calculated for tyrosine	59.7 per cent
H	»	6.37 » »	» » »	6.12 » »
N	»	7.75 » »	» » »	7.73 » »

The reticulocyte ripening activity of the original preparation 0—85 is described in a paper by PLUM (1942).

Table 2 shows the monomolecular constants k with varying concentrations of a preparation of H diluted with 0.9 per cent sodium chloride with and without 5 per cent of fraction D added.

Table 2.

Activation of the reticulocyte ripening power of preparation H with preparation D. The solution of H was made isotonic by adding sodium chloride.

k_s = monomolecular constant of spontaneous ripening.
k_f = » » » ripening induced by some factor.
$k = k_f \div k_s$.
k_s and k_f average of constants calculated after 2, 4 and 6 hours incubation.

Reticulocytes incubated at 40° with solutions containing:			k_s	k_f	k	per cent H	per cent D
c.c. H	c.c. D	c.c. 0.9 per cent NaCl					
0	0	2.0	0.0156	—	—	0	0
0.2	0	1.8	—	0.0210	0.0054	10	0
0.2	0.1	1.7	—	0.0422	0.0266	10	5
0.4	0	1.6	—	0.0214	0.0058	20	0
0.4	0.1	1.5	—	0.0543	0.0387	20	5

Fig. 1 gives an experiment showing the activating effect of the preparations D and DK. This experiment was carried out as described in the experiment given in Table 2 but here the ripening and activating substances to be tested were diluted with plasma instead of physiological saline solution. In some cases the blood cells have some tendency to aggregate. This is prevented by

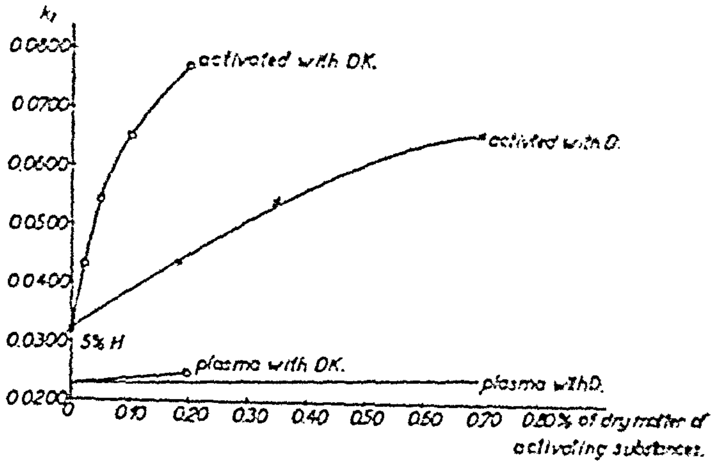

Fig. 1. Activation of 5 % H with increasing quantities of D and of DK in plasma.

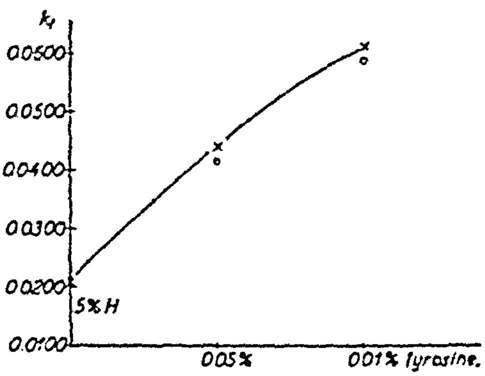

Fig. 2. Activating effect of synthetic tyrosine and tyrosine from liver extract.

× Activating effect of synthetic l-tyrosine
O Activating effect of tyrosine from liver extract

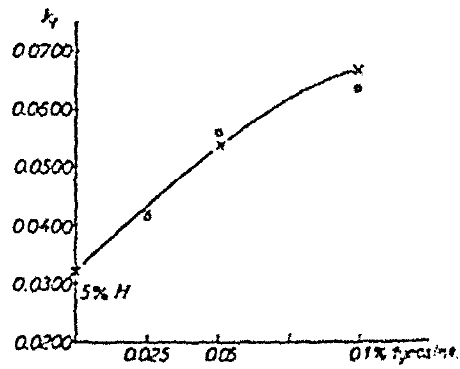

Fig. 3. Activation of 5 % H with fraction DK and with l-tyrosine.

O Activating effect of pure l-tyrosine
× Activating effect of DK based on the tyrosine content

using plasma as a suspending medium which makes the countings easier and more accurate. It is seen that the ripening substances in plasma are not activated by D and DK and that the butanol material extracted in this experiment yields on purification seven times the original activator used.

Fig. 2 shows that l-tyrosine, prepared in the described way from DK, has the same activating effect as synthetic l-tyrosine while fig. 3 proves that the tyrosine content in the preparation DK corresponds satisfactorily to the activating effect of the concentration found in this fraction. In these experiments too the blood corpuscles are suspended in plasma instead of in saline solution.

Summary.

The reticulocyte ripening principle in liver extracts consists of a thermolabile fraction which can be absorbed by floridin and a thermostable fraction which cannot be absorbed by floridin.

The latter fraction has only little effect by itself but activates the effect of the thermostable component.

The thermostable activating component is identical with l-tyrosine which is found in the liver extracts.

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From the Biol. Laboratories, Medicinalco, Copenhagen S.

Amino Acids and Tyrosine-like Substances as Activators of the Reticulocyte Ripening Principle.

By

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(Received 23 May 1942.)

In a previous paper (JACOBSEN and PLUM (1942)) we described the division of the reticulocyte ripening principle described by PLUM (1942) into at least two fractions, a thermolabile and a thermostable factor, the latter being identical with tyrosine.

In the present paper we give the results of investigations with other amino acids and various tyrosine-like substances in respect of their activating power in reticulocyte ripening. No other amino acid than tyrosine including phenylalanine possess any activating effect. Experiments with tyrosine derivatives show that the effect is not quite specific. A number of substances with a phenol group and an amino group in the side chain all show an activation. This configuration, however, is essential. If the OH group in the phenyl nucleus is displaced from the para to the meta position the effect has gone.

From these experiments we may conclude that even if an effect is found in some substances closely related to tyrosine, the activating power of this amino acid is rather specific.

Experimental.

The thermolabile fraction II was prepared as described in the paper by JACOBSEN and PLUM (1942). Liver extract was treated with floridin and the floridin eluted with liquid phenol which was dissolved in water and extracted with ether. The water

phase was then concentrated under reduced pressure to half the volume of the liver extract used. This preparation was added in an amount of 5 per cent to the solution in which the reticulocytes were suspended.

Table 1.

Testing of glycine.

Incubated in:			mg per cent glycine in incubation solution corrected by adding water or H.	Monomolecular constant of reticulocyte ripening	Increase in monomolecular constant due to adding glycine
ml H	Water	+ 1.9 ml 0.9 per cent NaCl mg per cent glycine in added salt solution			
0.00	0.10	0	0	0.0091	—
0.00	0.10	4.14	3.94	0.0104	0.0018
0.00	0.10	8.28	7.88	0.0113	0.0022
0.00	0.10	12.42	11.82	0.0120	0.0029
0.10	0.00	0	0	0.0218	—
0.10	0.00	4.14	3.94	0.0229	0.0011
0.10	0.00	8.28	7.88	0.0241	0.0023
0.10	0.00	12.42	11.82	0.0257	0.0039

A typical experiment was carried out as shown in Table 1. The concentrations of the amino acids were chosen so that they all had the same *molarity*. All the monomolecular constants are the average of three determinations after two, four, and six hours incubation at 40°.

The results of testing the amino acids investigated are collected in Table 2. All the experiments were performed as described in Table 1 but the details are omitted. The amino acids were mostly obtained from HOFFMANN LA ROCHE while a few were kindly supplied by Professor EINAR LUNDSGAARD. Their purity was controlled by titration which showed a purity of 99.0 to 99.5 per cent.

The tyrosine derivatives investigated are put together in Table 3. All the substances synthesised were prepared by one of us (E. J.) and used in a purity of 96 to 99 per cent. The experiments were carried out in a way similar to that described with the amino acids, but generally we used plasma as diluting liquid instead

Table 2.

Activating power of some amino acids.

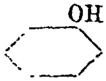
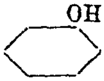
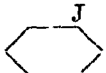
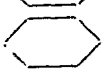
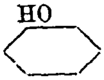
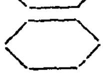
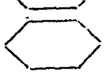
The letters a and b indicate isomolar solutions.

Amino acid	mg per cent amino acid in incubation solution		Increase in monomolecular constant due to the amino acid when added to	
			0.9 per cent NaCl	0.9 per cent NaCl with 5 per cent H
Glycine	3.94	a	0.0013	0.0011
»	7.88	b	0.0022	0.0023
»	11.82		0.0029	0.0039
d-Alanine	4.65	a	0.0025	0.0027
»	9.3	b	0.0032	0.0035
»	18.7		0.0056	0.0047
Cystine	12.83	a	0.0038	0.0035
»	25.65	b	0.0048	0.0047
»	51.3		0.0051	0.0053
dl-Valine	12.3	a	0.0018	0.0017
»	24.6	b	0.0033	0.0033
l-Leucine	13.8	a	0.0014	0.0012
»	27.6	b	0.0029	0.0027
Phenylalanine . . .	17.5	a	0.0028	0.0035
» . . .	35.0	b	0.0046	0.0075
l-Tyrosine	9.5		0.0020	0.0161
»	19.0	a	0.0033	0.0231
»	28.5		0.0048	0.0304
»	38.0	b	0.0056	0.0359
l-Tryptophane . . .	21	a	0.0021	0.0008
» . . .	42	b	0.0028	0.0030
l-Histidine	22.1	a	0.0021	0.0012
»	44.2	b	0.0035	0.0029
l-Proline	12.1	a	0.0017	0.0016
»	24.2	b	0.0051	0.0024
Asparagenic acid .	13.9	a	0.0011	0.0001
» » .	27.7	b	0.0032	0.0028
d-Glutaminic acid .	17.3	a	0.0012	0.0013
» » .	34.6	b	0.0025	0.0030
d-Lysine	21.9	a	0.0012	0.0009
»	43.8	b	0.0021	0.0025
Arginine	18.7	a	0.0010	0.0018
»	34.4	b	0.0025	0.0025

Table 3.

The tyrosine-like substances examined.

$++$ and $+$ = activation. 0 = no effect. $-$ = inhibition.

Name	Formula	Activating effect	Source
l-tyrosine . . . 181	HO  $CH_2CH(NH_2)COOH$	+ +	Hoffmann La Roche & Co. or from liver extracts.
dl-tyrosine . . . 181		+ +	Synthetic after ERLENMEYER and HALSEY (1899).
dl *o*-tyrosine . . 181	OH  $CH_2CH(NH_2)COOH$	0	Synthetic after JOHNSON and SCOTT (1915).
dl m-tyrosine . . 181	OH  $CH_2CH(NH_2)COOH$	+ −	Synthetic after BLUM (1908).
p-methoxyphenyl-alanine 195	CH_3O  $CH_2CH(NH_2)COOH$	0	Synthetic after the method of JOHNSON and SCOTT (1915).
p-o-y m-methoxy-phenylalanine . 212	OCH_3 HO  $CH_2CH(NH_2)COOH$	+	Synthetic after the method of JOHNSON and SCOTT (1915).
Dijodtyrosine . 433	J HO  $CH_2CH(NH_2)COOH$ J	+	Hoffmann La Roche & Co.
l-tyrosineethyl-ester 209	HO  $CH_2CH(NH_2)CO \cdot OC_2H_5$	+	Synthetic with HCl in alcohol.
Tyramine . . . 173	HO  $CH_2CH_2NH_2$, HCl	+	Hoffmann La Roche & Co.
Oxedrine tartrate 242	HO  $CHOH\ CH_2 \cdot NH\ CH_3$, $\frac{1}{2}C_4H_6O_6$	+	Dispensatorium Danicum.
β-p-oxyphenyl-isopropylmethyl-amine 214	HO  $CH_2CH(NHCH_3)CH_3$, $\frac{1}{2}H_2SO_4$	+	»Syncordan» MCO.
l-adrenaline . . 219	HO HO  $CHOH\ CH_2(NHCH_3)$, HCl	+	Pharmacopea Danica.
Phenylamine . . 165	 $CH_2CH(NH_2)COOH$	0	Hoffmann La Roche & Co.
β-phenylethyl-amine 167	 $CH_2CH_2NH_2$, $\frac{1}{2}H_2SO_4$	0	
p-oxyphenyl pyruvic acid . 168	HO  $CH_2CO \cdot COOH$	+	Synthetic after NEUBAUER (1909).
p-oxyphenyl lactic acid . . 170	HO  $CH_2CHOH \cdot COOH$	+	Synthetic after KOTAKE (1911).

of saline solution as this procedure made the countings much easier. The results of the ripening experiments with tyrosine-like substances are given in figs. 1, 2, 3, 4, and 5. In order to compare the substances investigated, figures showing equivalent amounts are plotted on the abscissa.

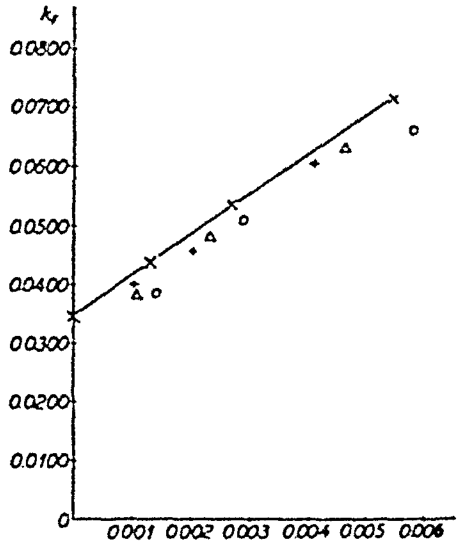


Fig. 1. Testing of tyramine, tyrosine-ethylester and oxedrin.

× tyrosine.
○ tyramine.
△ tyrosine-ethylester
+ oxedrin.

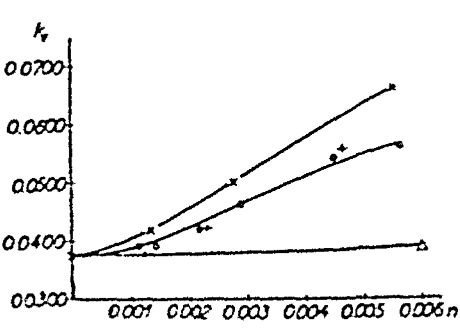


Fig. 2. Testing of tyramine, adrenalin, diiodotyrosine and β-phenylethylamine.

× tyrosine
○ tyramine
● l-adrenalin, HCl
+ diiodotyrosine
△ β-phenylethylamine

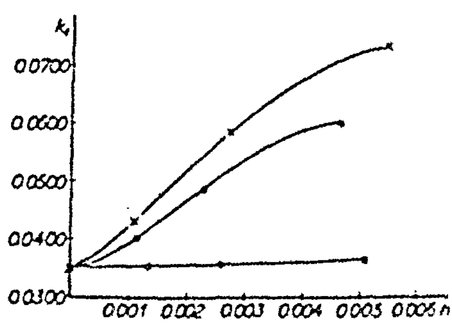


Fig. 3. Testing of 4-methoxyphenylalanine and 4-oxy-3-methoxyphenylalanine.

× tyrosine
○ 4-methoxyphenylalanine
● 4-oxy-3-methoxyphenylalanine

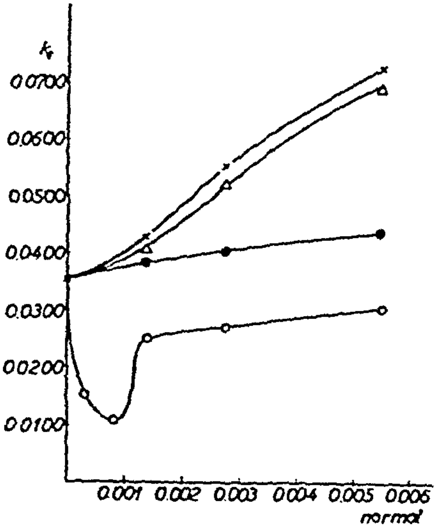


Fig. 4. Testing of racemic tyrosine, o-tyrosine and m-tyrosine.

× l-tyrosine
△ dl-tyrosine
● o-tyrosine
○ m-tyrosine

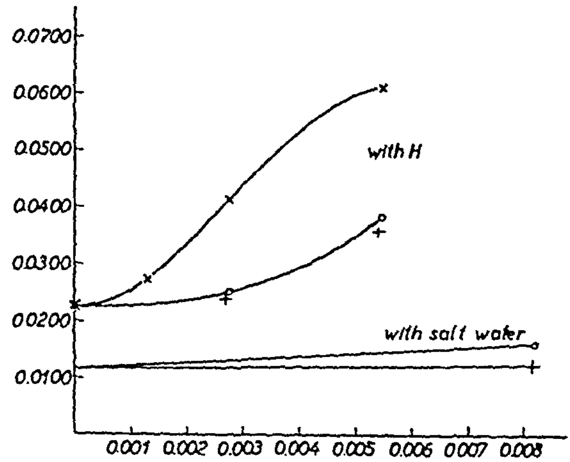


Fig. 5. Testing of p-oxyphenyl-pyruvic acid and p-oxyphenyl-lactic acid.

× tyrosine
○ p-oxyphenyl-pyruvic acid
+ p-oxyphenyl-lactic acid

Discussion.

None of the synthetic substances possess greater activating effect than the l-tyrosine. dl.-tyrosine has only slightly less effect than l-tyrosine. The d-tyrosine must thus have considerable activating power.

As to the side chain, the carboxyl group is desirable but not essential to maintain the effect. Both tyrosinethylester and tyramine activates fairly. It is difficult to decide if the amino group must be present or not. p-oxyphenylpyruvic acid and p-oxyphenyllactic acid are effective. It is, however, possible that these substances can be transformed into tyrosine in the blood cells.

The amino group can be exchanged with a methylamino group since both adrenalin and oxedrin (sympathol) show some effect. Nor does the induction of an OH group in the α position destroy the activating power.

In the nucleus, however, the OH group is essential as phenylalanine and p-methoxyphenylalanine show practically no activating power. The phenol group must be placed in the para position since o-oxy-phenylalanine (o-tyrosine) is practically quite without any activity.

It is very remarkable that m-oxyphenylalanine (m-tyrosine) not only possesses no activating effect but in some concentrations shows a considerable inhibition of the ripening of the reticulocytes. A further investigation of this phenomenon will be treated in a forth-coming paper. Finally our present experiment shows that the 2nd and the 6th positions of the nucleus can be blocked by iodine without complete loss of the activating effect. When the hydrogen in the 3rd position is replaced by a methoxyl group or by an oxy group (as found in adrenalin) the effect is only diminished but still present.

Since at present we know nothing about the mechanism of the induced ripening of the reticulocytes, it is too early to discuss the significance of the present findings.

It is possible that the tyrosine may be transformed into some indolin-derivative during there action with the reticular substance, a reaction similar to that which happens when tyrosine is transformed into melanin. So far nothing speaks against this assumption, but the discussion must be postponed until further experimental results are available.

Summary.

None of the 13 most common amino acids show any activating effect on the reticulocyte ripening principle. Only tyrosine has this effect. Investigations on some tyrosine derivatives show that the phenol group is essential to the effect of tyrosine. The phenol group must be in para position to the side chain. The amino group can be replaced by an oxo-, an oxy-, or a methylamino group. Minor changes in the molecule not affecting the OH group give only some reduction in the activity.

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A Colorimetrical Carbon-monoxide-hemoglobin Method of Determination for Clinical Use.

By

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(Received 4 June 1942.)

Carbon monoxide poisoning in one shape or another is one of the commonest forms of poisoning — perhaps, indeed, even the commonest. A large number of carbon-monoxide-hemoglobin methods of determination for clinical use have therefore been worked out, of which the colorimetric methods have been those most used. (RAY, BLAIR and THOMAS (1932), LUSZCZAK (1936), MAY (1937), OETTEL (1938 a and b), MATTHES and GROSS (1939), MAY (1939 a and b) and HEILMEYER (1933)).

Certain demands must be made of a good colorimetric, clinical HbCO method of determination. It must

1) be easy to perform;

2) be practicable at any hospital at all without calling for any particularly elaborate extra apparatus;

3) preferably be a micro-method so that samples may be taken from the tip of the finger or the lobe of the ear and venous puncture may be avoided.

The majority of methods fulfil the above requirements.

As appears from fig. 1, however, a certain amount of CO is dissociated from the CO-hemoglobin when the blood is diluted, and the value obtained will thus be too low. All the methods listed in the references work with strongly diluted blood. The authors have accordingly worked out a method in which this source of error is avoided by means of making colorimetric determinations on the blood in very thin cuvettes, it being thus

possible to dilute so slightly that the error arising from the dilution may be obviated. As reading-instrument a Stufen-photometer may be used, as it will be found in most larger hospitals. Greater accuracy could, naturally, be achieved by using a spectral photometer instead. And it is of course easy to adapt the method for this apparatus if required.

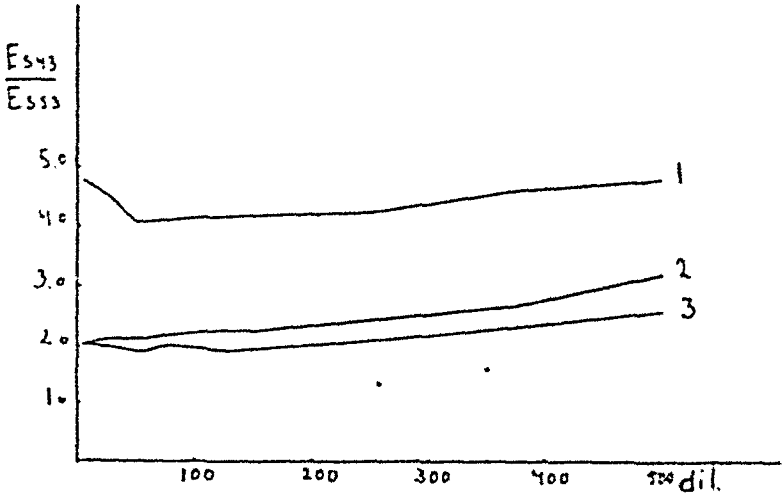

Fig. 1. Curve 1: HbCO-saturated blood is diluted up to 500 times; $Na_2S_2O_4$ is added, followed by a colorimetric determination (curve 2). Curve 3 shows the same thing, but with renewed bubbling with CO after the dilution.

The principle: The pigments occurring in the blood — Hb, HbCO, HbO_2 and possibly MetHb are translated with reducing agents to two — Hb and HbCO. The percentage of HbCO in total-Hb can then easily be determined by Heilmayer's elaboration of Vierordt's method (HEILMEYER, 1933). According to the expression:

$$X = 100 \cdot \frac{\frac{1}{B} - Q \cdot \frac{1}{b}}{Q \cdot \frac{1}{a} - \frac{1}{A} + \frac{1}{B} - Q \cdot \frac{1}{b}};$$

where X = the desired %-content of the one pigment, in this case HbCO.

A = absorption-relation = $\frac{\text{concentration}}{\text{extinction coefficient}}$ for HbCO at the wave-length λ_1.

a = absorption-relation = $\frac{\text{concentration}}{\text{extinction coefficient}}$ for HbCO at the wave-length λ_2.

B = absorption-relation = $\frac{\text{concentration}}{\text{extinction coefficient}}$ for Hb at the wave-length λ_1.

b = absorption-relation = $\frac{\text{concentration}}{\text{extinction coefficient}}$ for Hb at the wave-length λ_2.

$Q = \frac{E_1}{E_2}$, where E_1 and E_2 represent the extinctions at λ_1 and λ_2, we can draw a curve with %-content HbCO and Q on the axes.

The extinctions for Hb and HbCO are determined in a cuvette that will be described below. The thickness of the layer is thus constant. As, further, [Hb] and [HbCO] are of equal magnitude, since the samples have been taken from the same experimental subject and equally diluted, we can substitute for $\frac{1}{A}$, $\frac{1}{a}$, $\frac{1}{B}$ and $\frac{1}{b}$ $E_{HbCO_{\lambda 1}}$, $E_{HbCO_{\lambda 2}}$, $E_{Hb_{\lambda 1}}$ and $E_{Hb_{\lambda 2}}$ respectively.

The greater the difference between Q_{HbCO} and Q_{Hb}, the more accurate will be the result. An investigation of the values that WARBURG, CHRISTIAN and NEGELEIN obtained spectro-photometrically gives the following: the γ-band for HbCO lies at 420 mμ, for Hb at 430 mμ. These values are combined with the low absorption-values at alternatively e. g. 510 and 530 mμ. (Stufen-filters S 51 and S 53 are generally available).

The values of β (the "molar absorption-coefficient") are given multiplied by 10^{-8}

	HbCO	Hb
$\frac{\beta_{420}}{\beta_{510}}$	$\frac{4.84}{0.16} = 30.25$	$\frac{2.75}{0.12} = 22.92$
$\frac{\beta_{430}}{\beta_{510}}$	$\frac{2.11}{0.16} = 13.10$	$\frac{3.23}{0.12} = 26.92$
$\frac{\beta_{420}}{\beta_{530}}$	$\frac{4.84}{0.30} = 16.13$	$\frac{2.75}{0.18} = 15.28$
$\frac{\beta_{430}}{\beta_{530}}$	$\frac{2.11}{0.30} = 7.03$	$\frac{3.23}{0.18} = 17.94$

The greatest difference between the quotients for HbCO and Hb is thus obtained by employing the wave-lengths 430 mμ and 530 mμ. True, these figures were obtained with spectrophotometer values; but our determinations show the conditions for the Stufen-photometer to be about the same. The most suitable filters in the Stufen-photometer are thus S 43 and S 53, corresponding to λ_1 and λ_2 respectively in the formula.

Apparatus and solutions:

The sample is taken with a pipette as shown in fig. 2. From the tip to the first division it should hold approximately 0.08

Fig. 2. Dilution pipette.

ml, in any case not over 0.1 ml: From the first division to the second the volume is twice as great, thus about 0.16 ml. One thus obtains a dilution of the blood in the ratio 1:3. The tip of the pipette must be very fine. An outer diameter of 0.6—0.8 mm. seems to be suitable. If it is larger the out-flowing fluid will be unnecessarily exposed to the air.

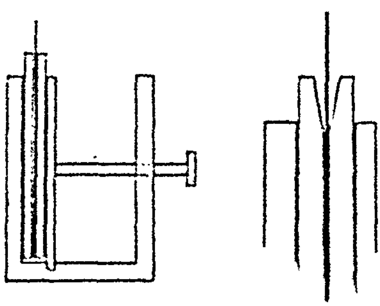

Fig. 3. Section of cuvette.

The cuvette (fig. 3) consists of two plane-parallel, polished glass slides, 25 × 35 × 2 mm., separated by two bands of cold-rolled and tempered steel of factory-controlled thickness. The most suitable thickness has been found to be 0.10 mm. If one uses 0.03—0.04 mm. it is, certainly, possible to carry out colorimetric determinations on undiluted blood, but with this thickness we found it difficult to get constant values for Q_{Hb} (on a couple of occasions with extremely low values of Q — about 4.20 instead of 4.82 — one could see weak absorption-bands from HbO_2 in the spectroscope). The glass slides are provided at the top with twin bevelling that forms a little groove into which the tip of the pipette is inserted so that there is the least possible exposure to the air. The "cuvette" is held together with two ordinary screw-clamps cased in rubber.

A similar technique as regards cuvettes has earlier been employed by BUTTERFIELD (1912).[1]

As diluting fluid we used:

Sodium citrate. 2 aq	0.3 g
Saponin	0.3 g
Sodium hydro-sulphite. 2 aq	0.5 g
Buffer (4 vols. m/15 prim. + 6 vols. m/15 sec. phosphate, pH 6.97) to 10.0 ml.	

The solution is buffered in order to prevent SO_2 formed by oxidation of $Na_2S_2O_4$ from sinking the pH too much, which would mean that stromatin might be precipitated.

It may here be mentioned that the same tubes that are used for blood-tests in alcohol-determinations ("Widmark tubes") (WIDMARK 1922) may be used for samples for carbon monoxide determinations. Such a tube holds about 0.12 ml. The blood is sucked over in the pipette via a centimeter-long rubber ligature. The taking of samples and sending of same to the laboratories can be effected in precisely the same way in both cases, a point that is probably of value from the psychological point of view, as it has sometimes proved troublesome to get blood-tests from motorists in case of traffic-accidents where the influence of alcohol is suspected.

Method: From the tip of the finger, the lobe of the ear or a "Widmark tube" blood is sucked up to the first division, diluting fluid to the second division, after which the liquids are mixed by sucking backwards and forwards between the two 'bulbs' of the pipette. When the tip is then placed in the previously mentioned groove, the solution is sucked in to a capillary layer where it is protected from air and from drying. $E_{S_{43}}$ and $E_{S_{53}}$ are then determined, the quotient $\frac{E_{S_{43}}}{E_{S_{53}}}$ is formed and the desired value is obtained from the curve.

[1] It is interesting to note that BUTTERFIELD, without giving any reason for this, does not use a lesser dilution of the blood than 1:3, although he tried to work with as concentrated solutions as possibles.

Results.

Hb

	E_{543}	E_{553}	Q
	1.267	0.260	4.87
	1.254	0.261	4.81
	1.250	0.262	4.77
Average:	1.257	0.261	4.82

HbCO

	E_{543}	E_{553}	Q
	0.747	0.367	2.04
	0.749	0.365	2.05
	0.760	0.370	2.05
Average:	0.752	0.367	2.05

HbCO was prepared here and in the following experiments in the same way: a mixture of 49 vols. of N_2 and 1 vol. of CO, prepared in the ordinary way from formic acid, was bubbled through blood for 20 minutes. By using diluted carbon monoxide the effect of physically dissolved CO was avoided in the experiments described in the following. Experiments with blood from different healthy experimental subjects have given the same result. On the basis of these values a table was made up with assumed, varying values of Q.

Q	%-content of HbCO
4.82	0
4.60	5.5
4.40	11
4.20	17
4.00	23
3.80	29
3.60	36
3.40	43
3.20	50
3.00	58
2.80	66
2.60	74
2.40	83
2.20	92
2.05	100

Control experiment: The HbCO-content was determined in samples with a known proportion of HbCO. HbCO was prepared in the way already described.

cc HbCO-solut.	cc HbO_2-solut.	$E_{S_{43}}$	$E_{S_{53}}$	$\frac{E_{S_{43}}}{E_{S_{53}}}$	Calc.%	Empir.%	Deviat.
0.05	0.45	1.29	0.292	4.42	10	10.5	+ 0.5
0.05	0.45	1.28	0.290	4.41	10	10.5	+ 0.5
0.05	0.45	1.30	0.300	4.33	10	13	+ 3
0.10	0.40	1.20	0.300	4.00	20	23	+ 3
0.20	0.80	1.28	0.316	4.05	20	21.5	+ 1.5
0.10	0.40	1.16	0.282	4.11	20	20	0
0.10	0.40	1.38	0.346	3.99	20	23.5	+ 3.5
0.125	0.375	1.34	0.340	3.94	25	25.5	+ 0.5
0.15	0.35	1.29	0.343	3.76	30	31	+ 1
0.15	0.35	1.15	0.295	3.90	30	26.5	— 3.5
0.10	0.20	1.16	0.310	3.74	33.3	31.5	— 1.8
0.20	0.30	1.29	0.386	3.37	40	44	+ 4
0.20	0.30	1.28	0.372	3.44	40	41.5	+ 1.5
0.20	0.30	1.11	0.314	3.54	40	38	— 2
0.25	0.25	1.06	0.324	3.27	50	48	— 2
0.50	0.50	1.26	0.317	3.17	50	51.5	+ 1.5
0.30	0.20	1.08	0.371	2.90	60	61	+ 1
0.35	0.15	1.16	0.435	2.67	70	67.5	— 2.5
0.40	0.10	0.98	0.395	2.46	80	80.5	+ 0.5
0.45	0.05	1.05	0.477	2.20	90	92.5	+ 2.5

We also carried out determinations parallel with a method described by PAUL and WRETLIND (1942), based upon precipitation of the reduced hemoglobin under certain conditions, while HbCO remains in solution.

$E_{S_{43}}$	$E_{S_{53}}$	Q	% HbCO	Accord. PAUL & WRETLIND	Difference
1.23	0.265	4.64	4.5	7	2.5
1.24	0.266	4.66	4	6	2.0
1.02	0.250	4.08	20.5	23	2.5
0.99	0.241	4.07	21	27	6
1.08	0.272	3.97	24.5	23	1.5

Serum from a jaundice patient with Meulengracht-value 1: 60 gave $S_{43} = 0.05$ and $S_{53} = 0.01$. A blood-sample from a patient with chronic myelosis, fully able to work and complaining only of a slight tenderness in the abdomen, and with 64 % Hb, 3.4 mill. red and 225,000 white blood corpuscles per cubic millimeter gave QHb = 4.86 and QHbCO = 2.06. One may thus abstract from these possible sources of error.

Summary.

Carbon-monoxide-hemoglobin methods of determination that deal with strongly diluted blood must give too low values owing to the fact that carbon-monoxide-hemoglobin dissociates on dilution. A simple method, sufficiently accurate for clinical use, has been worked out from which these sources of error have been eliminated.

The determination is easy to carry out and takes only a few minutes. An important advantage of the method is that it enables determination on a micro-scale, so that blood may be taken from the tip of the finger. Samples can be sent in capillary tubes, as for instance is done in the case of blood-tests for alcohol according to Widmark's method.

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Measurement and Properties of Antithrombin.

By

TAGE ASTRUP and SVEN DARLING.

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As is well known, plasma and serum contain substances which inactivate thrombin. The inactive product formed is by most authors called metathrombin and thought to be the substance which by treatment with alcali or acid can be reactivated into thrombin (cf. GASSER (1917)). The problem of the relationship between thrombin, antithrombin, metathrombin and the reactivation of the last mentioned substance has however not yet been solved, and the opinions of most authors are quite contradictory (cf. WÖHLISCH (1929, 1940)).

The purpose of this paper is to investigate the normal antithrombin of serum and plasma and its relation of thrombin. The question of metathrombin falls beyond the scope of the work, and the antithrombin formed by addition of heparin is treated in a subsequent paper.

Investigations on antithrombin have been carried out by several authors, but the work has mainly been on the qualitative line, and only few have tried to measure the amount of antithrombin quantitatively. J. MELLANBY (1909) already showed that the amount of antithrombin in plasma could inactivate many times the amount of thrombin formed from the same amount of plasma. This was confirmed by GASSER (1917).

Experimental.

1. The Measurement.

The ox *thrombin* used was prepared as before (ASTRUP and DARLING (1940, 1941 a)). For the determinations, ox fibrinogen as described by ASTRUP and DARLING (1942), was used.

Oxalated ox plasma was used as antithrombin-containing material. From this serum was prepared after one of the following three methods: 1). Recalcification of the plasma. 2). Removal of fibrinogen by coagulation with a little thrombin. 3). Denaturation of fibrinogen by heating to 56° in a water-bath for 3 minutes and removal of the precipitate.

Smaller amounts of material were usually prepared by heating, and larger amounts by recalcifying. The thrombin formed under the recalcification is negligible in proportion to the amount of thrombin, which can be inactivated by the antithrombin in the plasma, and it is therefore not necessary to take it into consideration. The same applies to the addition of the small amount of thrombin necessary for clotting the fibrinogen in the second method.

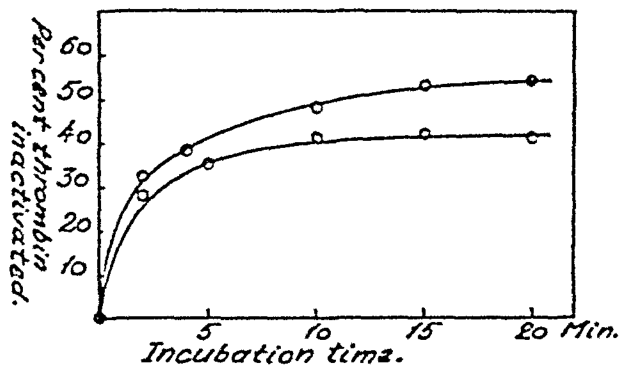

Fig. 1. Per cent thrombin inactivated after incubation with ox serum.

Our unit for antithrombin is based on our unit for thrombin (ASTRUP and DARLING (1940, 1941 a, 1942), cf. also ASTRUP (1941)). An antithrombin unit (A. T. U.) is the amount of antithrombic substance which can neutralize a unit of thrombin (T. U.).

For the determinations a thrombin solution of known strength is incubated with the antithrombin-containing material until saturation and the amount of thrombin left is measured. The incubation is carried out at 37° as the neutralization proceeds only slowly at lower temperatures (COLLINGWOOD and MAC MAHON (1914)).

For determination of the incubation time the following experiment is made:

To a thrombin solution containing 41 T. U. per ml, 0.10 ml of serum prepared by recalcification is added for every ml of thrombin solution. The mixture is incubated at 37° and the course of the reaction is followed by determination of the thrombin left after varying reaction times. The measurements are made as described before (ASTRUP and DARLING (1942)) by placing 0.10 m of the solution in a clotting tube and adding 1.0 ml of a fibrinogen solution, whereupon the tube is placed in a water-bath at 37° and the clotting time noted as the time for formation of the first threads of fibrin. From the clotting time of the experiment and the clotting time of a known control solution,

with physiological NaCl added instead of serum, the content of thrombin is determined as usual. The amount of thrombin inactivated is then found and expressed in per cent of the original content. In this way the two curves in Fig. 1 are found. Fig. 1 shows that the neutralization of thrombin by normal antithrombin proceeds rather slowly and is only complete after a lapse of about 15 minutes. For determination of antithrombin it is therefore necessary to incubate the mixture for at least 15 minutes at 37°, a condition which has not been fulfilled by several authors (see later).

For the determination of an unknown amount of antithrombin it is further necessary to know the relation between the amount of antithrombin added and the amount of thrombin neutralized. Only if

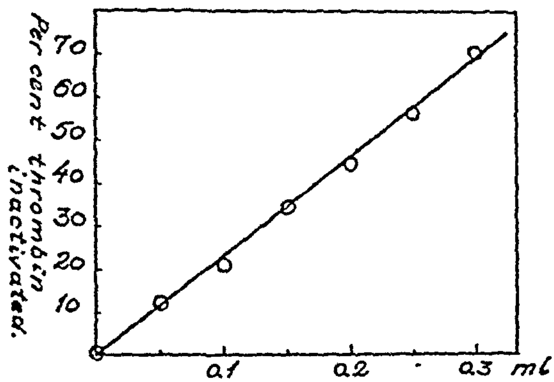

Fig. 2. Inactivation of thrombin by increasing amounts of ox serum.

there is a simple relationship between these quantities is it possible to devise a simple method for measuring antithrombin. The following experiment shows that there is a direct proportionality between the antithrombin added and the thrombin inactivated under the experimental conditions chosen.

To 7 tubes containing 1 ml thrombin solution, (41 T. U. per ml) 0.00, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 ml of serum is added and then physiological NaCl until the total volume of every sample is 1.30 ml. The mixtures are incubated at 37° for 15 minutes and the thrombin content is then determined as usual. The thrombin inactivated is then expressed in per cent of the original content, and a curve is drawn with the amount of antithrombin (serum) added in ml as abscissa and the amount of thrombin inactivated in per cent as ordinate. Such a curve is shown in Fig. 2.

The curve shows that there is a linear relation between the amount of antithrombin added and the amount of thrombin inactivated. It is seen than 0.2 ml of the serum used inactivates 46 per cent of the thrombin added, *i. e.* 46 per cent of 41 T. U. One ml of serum hence inactivates $\frac{41 \cdot 46}{100 \cdot 0.2}$ T. U. equal to 94 T. U. which therefore is the content of antithrombin units per ml of the serum in question.

Based on these experiments and on later experiences the measurement of antithrombin is best carried out as follows:

Of a thrombin preparation containing about 12 T. U. per mg of substance, 50 mg are dissolved in 15 ml of physiological NaCl or a diluted diethylbarbiturate buffer with pH 7.4, as prepared by THORDARSON (1940). Phosphate buffer was found to be inconvenient. One drop of octyl alcohol is added and after standing for one hour the solution is centrifuged or filtered. There results a solution containing about 40 T. U. per ml. The content per ml is called a. Most of the thrombin used in this work was obtained from "Lovens kemiske Fabrik", Copenhagen.

The fibrinogen used for the measurements was precipitated with ammonium sulphate from oxalated ox plasma as described by ASTRUP and DARLING (1942). It was diluted with physiological NaCl or diethyl barbiturate buffer, and distilled water was added until a good reactivity was obtained, as the reaction velocity is to a high degree dependent upon the salt concentration (ASTRUP (1941)). The clotting time should be about 10 seconds by addition of 1 ml fibrinogen solution to 0.10 m of the original solution of thrombin. The fibrinogen solution is placed in ice-water during the experiments. After dilution it has only a moderate stability.

In a series of tubes antithrombin-containing material is placed in amounts ranging from 0.00, 0.05, 0.10, 0.15, to 0.20 ml, and physiological NaCl is added until the total volume in every tube is 0.3 ml. Then 1.0 ml of the thrombin solution, containing a T. U. is added to each tube. The tubes are placed in a water-bath at 37° for 15 minutes and are then transferred to ice-water. For every sample the thrombin content is determined by placing 0.10 ml of the incubated mixture in a clotting tube and measuring the clotting time after the addition of 1.0 ml of the fibrinogen solution in a transparent water-bath at 37°. Three determinations are as a rule made from every sample.

The control sample containing only thrombin solution and physiological NaCl, *i. e.* the first mentioned, gives the clotting time t_c. The amount in ml of antithrombin solution is called n, and the corresponding clotting time t_n. t_c corresponds to a T. U. in the mixture, and t_n therefore corresponds to $\frac{a \cdot t_c}{t_n}$ T. U. Originally there was a T. U., and hence $\left(a - \frac{a \cdot t_c}{t_n}\right)$ T. U. are inactivated, which corresponds to the amount of antithrombin units contained in the n ml antithrombic material in the sample. The content c of antithrombin in this material expressed in A. T. U. per ml is therefore obtained from the following equation (1):

$$c = \frac{a}{n}\left(1 - \frac{t_c}{t_n}\right) \text{A. T. U. per ml} \quad \ldots \ldots \ldots \quad (1).$$

In order to increase the accuracy of the determination, c is as a rule *not found directly from the measurements.* Instead, a curve is drawn with the incubated amount of antithrombic material as ml

added as abscissa, and the corresponding value of $\frac{t_o}{t_n}$ as ordinate. From the straight line obtained, a corrected value of $\frac{t_o}{t_n}$ corresponding to a given amount n of antithrombic material is found by interpolation. These values are then put into equation (1) and c determined. In this way the accuracy of the antithrombin determination is increased so much, that the deviation is only about ± 5 per cent. The determination of the clotting time proper gives an experimental deviation of 5 to 10 per cent.

Table 1.

Thrombin solution ml	Plasma ml	Physiol. NaCl ml	t Determinations sec.	t mean sec.	$\frac{t_o}{t_n}$
1.00	0.00	0.30	11.0, 11.2, 11.0	11.1	1.00
1.00	0.05	0.25	14.4, 15.0, 14.6	14.7	0.76
1.00	0.10	0.20	17.8, 18.2, 17.8	17.9	0.62
1.00	0.15	0.15	23.6, 24.4, 24.6	24.2	0.46
1.00	0.20	0.10	39.2, 39.0, 40.0	39.4	0.28

The following is an example of such a determination. Rabbit plasma obtained from citrated blood (in the proportion 9 ml blood to 1 ml of 3.5 per cent sodium citrate solution) is used. By mixing the thrombin solution and the antithrombin-containing plasma the fibrinogen is immediately transformed into fibrin. This, however, does not interfere with the antithrombin determinations as only the clear fluid is used. The experimental data are seen from Table 1.

From these determinations the curve shown in Fig. 3 is drawn in the manner described before. It is seen that the points lie on a straight line with a sufficient accuracy. Only the determination for the addition of 0.05 ml plasma is too low, a fact which is very often met with. On the other hand, the value for 0.20 ml plasma often shows a tendency to be too high.

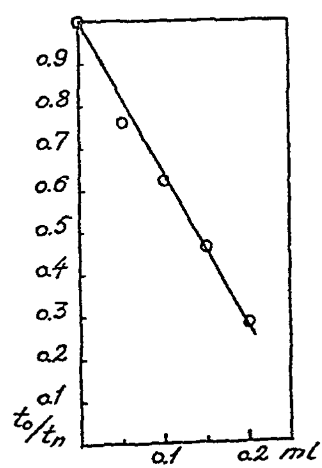

Fig. 3. Determination of antithrombin content of rabbit plasma.

From the curve the value for 0.20 ml plasma is found to be 0.28, which in

this case is in accordance with the experimental determination. By using equation (1) and putting $a = 41$ T. U. per ml and $n = 0.20$ ml, c is found to be 148 A. T. U. per ml in citrated rabbit plasma.

2. Antithrombin in Serum.

The antithrombin content of ox serum prepared by heating to 56° and fractions obtained by precipitation with ammonium sulphate is now investigated. The measurements of the solutions prepared are carried out as described in the preceding part.

30 ml of serum are diluted with 30 ml of distilled water and precipitated by dropwise addition of 60 ml of saturated ammonium sulphate under stirring. The precipitated globulin is removed by centrifugation and washed with 25 ml of 0.5 saturated ammonium sulphate. It is then dissolved in water and dialysed in a cellophane casing against tap water. After dialysing, 0.1 volume of 9 per cent NaCl is added to the solution. The solution is called G—I and contains 25 A. T. U. per ml. The volume is 50 ml. Total amount 1,250 A. T. U.

The centrifugate and the washing from G—I are united and saturated with solid ammonium sulphate. The albumin precipitated is removed by filtration, dissolved in water, dialysed and 9 per cent NaCl is added as before. The solution is called A—I. The volume is 25 ml and it contains 56 A. T. U. per ml. Total 1,400 A. T. U.

The serum used contained 93 A. T. U. per ml, total 2,790 A. T. U. One half of the antithrombin is thus found in the albumin fraction A—I and 45 per cent in the globulin fraction G—I. Total yield 95 per cent.

The globulin solution G—I is further fractionated by addition of one volume of saturated ammonium sulphate. The resulting precipitate G—II is isolated as before. The volume is 25 ml, containing 3 A. T. U. per ml. Total 75 A. T. U.

From the centrifuge and washing from G—II the albumin A—II is isolated. Volume 25 ml, contai ing 36 A. T. U. per ml. Total 900 A. T. U. Of the antithrombin content in G—I thus 72 per cent is found in the albumin fraction.

By reprecipitating G—II a further globulin G—III is obtained. Volume 10 ml containing 1 A. T. U. per ml. Total 10 A. T. U.

From these experiments it is seen that the antithrombin contained in serum is found in the albumin fraction, and that the globulin fraction contains practically no antithrombic substance after a few reprecipitations. The substance does not pass through a dialysing membrane. These results are thus in accordance with the experiences of most authors.

3. Antithrombin in Plasma.

For a more thorough investigation plasma is used as starting material. As it is found, that the antithrombin is not removed by treatment with tricalcium phosphate, the resulting prothrombin-free BORDET plasma can be used. For the following experiments therefore the centrifugate resulting from the preparation of fibrinogen is used for the measurements (ASTRUP and DARLING) (1942)). For the fractionation the principles described by HEWITT (1936) are used.

To 200 ml of BORDET plasma (ox), 300 ml of distilled water are added and then the fibrinogen is precipitated with 200 ml of saturated ammonium sulphate. 265 ml of saturated ammonium sulphate is added to the centrifugate (volume 620 ml), after which the degree of saturation is 0.5. The precipitated globulin is removed by filtration, dissolved in water and dialysed against physiological NaCl. The resulting solution is called A.

To the mother liquor from A (volume 830 ml) 1.6 ml of acetic acid are added. The precipitate is removed, dissolved in water and dialysed against tap water. The solution is called B.

To the mother liquor from B (volume 800 ml) 343 ml of saturated ammonium sulphate are added. Saturation degree 0.65. The precipitate is isolated as before and the resulting solution called C.

All these solutions are now analysed. The plasma used contained 120 A. T. U. per ml. Total 24,000 A. T. U. In Table 2 the results are shown.

Table 2.

Solution	Volume ml	A. T. U. per ml	mg N per ml	A. T. U. per mg N	A. T. U. total	Yield per cent
A . . .	65	22	7.64	2.9	1430	6
B . . .	55	1.2	6.22	0.2	66	0.3
C . . .	44	133	5.22	25.5	5850	24

From Table 2 it is seen that also in this case the antithrombin is found in the albumin fraction, and that a practically inactive fraction (B) can be precipitated with acid, while the antithrombin remains in solution (C). The yield, however, is not so large as in the preceding experiments with serum. As a rule the yield of antithrombin in fraction C ranges between 20 and 35 per cent of the content in the plasma used. It is possible, according to the experiments by VOLKERT (1941), that the antithrombin of plasma consists of two components, the greater part is the nor-

mal antithrombin and a smaller portion is an antithrombin formed from heparin. Thus it is possible to explain the presence of the two separated fractions A and C.

Fraction C is now used for further investigations. The following fractions are prepared:

To 10 ml of C, 15 ml of saturated ammonium sulphate are added, giving a saturation degree of 0.60. The mixture develops a slight turbidity, and 0.10 ml 1-m acetic acid is added. The precipitate formed is removed, dissolved in water and dialysed. Fraction D.

To the mother liquor from D 3.5 ml of saturated ammonium sulphate are added. Saturation degree 0.65. The precipitate gives the solution E.

To the mother liquor, resulting from E, 4.8 ml of saturated ammonium sulphate are added. Saturation degree now 0.70. The precipitate gives the solution F. Addition of more ammonium sulphate to the mother liquor from F gives no precipitation.

The resulting solutions are analysed and compared with the original C-fraction, which in this case is not so active as the C-fraction mentioned before. The results are shown in Table 3.

Table 3.

Solution	Volume ml	A. T. U. per ml	mg N per ml	A. T. U. per mg N	A. T. U. total	Yield per cent
C . . .	10	65	5.39	12	650	—
D . . .	6.5	13	1.45	9	85	13
E . . .	7.0	34	2.35	14.5	238	37
F . . .	3.0	9	0.39	23	27	4

The total yield by the fractionation was thus 54 per cent of the content in fraction C. It is seen that it is possible to divide C in smaller fractions with different contents of antithrombin. This seems to indicate that antithrombin is a definite substance and not the serum albumin as such — which is the opinion of several authors. However, it is to be pointed out that the activity of the antithrombin fractions is only very low in comparison with the activity of thrombin solutions. While the most active antithrombin fractions contained only about 25 A. T. U. per mg N it is easy to prepare thrombin preparations having over 800 T. U. per mg N (ASTRUP and DARLING) (1941 a)). Therefore, it is impossible to say anything about the probability of antithrombin being one definite, specific substance. In the preparation of thrombin a very good purification was obtained by

precipitation with acetone, but antithrombin is completely destroyed by treatment with acetone.

Some further properties of antithrombin will be discussed in a subsequent paper concerning the relation between antithrombin and heparin.

Discussion.

Already ALEXANDER SCHMIDT himself (1892) knew that in blood or serum thrombin gradually loses its clotting power. The presence of thrombin-inactivating substances was soon confirmed by BORDET and GENGOU (1904), MORAWITZ (1904 a, b) and MURASCHEW (1904). The presence of an antithrombic substance was concluded from the facts that plasma was clotted more slowly than a fibrinogen solution by the same amount of thrombin, and that thrombin gradually deteriorates in serum. It was found that the substance was relatively thermostable and can withstand heating to 58° for 15 min., as such heated plasma increases the clotting time of a fibrinogen solution.

Already some years before, BORDET and GENGOU (1901) had found an antithrombic substance in serum from guinea-pigs immunized with rabbit serum, and they were of the opinion that an immune antithrombin had been formed. This problem has only recently been solved by VOLKERT (1941 and to be published), and is found to be an unspecific reaction.

The method used by BORDET and GENGOU (1901) is to mix 0.1 ml of fresh rabbit serum (containing thrombin) with 0.6 ml of guinea-pig serum heated to 58° (containing antithrombin) and then to add 0.3 ml of goose plasma (as fibrinogen source). Serum from the treated guinea-pigs is found to inhibit coagulation the most.

The antithrombin present in peptone plasma, which later has been shown to be heparin, was already known by these authors to be a different substance from that present in normal blood and serum (MORAWITZ (1904 a, b, 1905)).

Since the days of MORAWITZ, the question about the nature of antithrombin and its rôle in blood clotting has been an unsolved problem (see f. i. the excellent reviews by WÖHLISCH (1929, 1940)), and the most different views have been expressed by various authors. Therefore, in the following only results of interest in connection with the present studies will be discussed.

MELLANBY (1909) was the first to show that antithrombin is present in large excess in comparison with the amount of thrombin which can be formed from plasma. Acetone destroys antithrombin. He was also the first to note that the neutralization proceeds at a relatively slow rate, so it is necessary to incubate (30 min. at 30°) the mixture before determining the activity. His experiments are carried further by COLLINGWOOD and MAC MAHON (1913, 1914). While MELLANBY worked with chicken plasma the same results were now obtained with human plasma.

From the work of these authors it is evident, that for an exact determination of the action of the antithrombic material present in normal plasma and serum it is absolutely necessary that the measurement shall be preceded by an incubation in order to complete the reaction. This has not been the case in the experiments of earlier investigators, and they have only succeeded because their thrombin solutions showed a low activity with a resulting long clotting time of the mixture, which gave the antithrombin an opportunity to act to some degree. This is also the case in the experiments by LANDSBERG (1913).

A prominent rôle in the blood clotting was assigned to the antithrombin through the theory of HOWELL (cfr. 1935), and very extensive investigations on the substance have been carried out by HOWELL and his pupils. In their first experiments no incubation was employed (RETTGER (1909), HOWELL (1912), WHIPPLE (1912, 1913) and WEYMOUTH (1913)).

In some of his experiments the last mentioned author uses an incubation time varying from some minutes to several hours. However, it was HOWELL himself (1914 a, b) who first described a procedure with incubation for 15 min., which may be called the first method for the determination of antithrombin. Subsequently this method was used by several authors (HOWELL (1915), DENNY and MINOT (1915), DRINKER and DRINKER (1915), HURWITZ and DRINKER (1915), MINOT (1916), MINOT, DENNY and DAVIS (1916), RICH (1917) and GASSER (1917)), trying to solve different physiological problems concerning antithrombin. The results are not satisfactory, however, as the accuracy of the method was slight. Later experiments by VOLKERT (1941 and unpublished) using our method could not confirm all the results obtained by those authors.

In HOWELLS method the antithrombin-containing plasma or serum is heated to 60° for 10 min. to destroy thrombin and pro-

thrombin. A little of the solution (one drop) is then incubated with varying amounts of thrombin solution (2 to 6 drops) for 15 min. at 37°, and the activity of the remaining thrombin is determined by means of a fibrinogen solution. As a rule the clotting time amounts to several minutes. The measurement is described especially by HOWELL (1914 b), DRINKER and DRINKER (1915), MINOT, DENNY and DAVIS (1916) and GASSER (1917), and from these investigations it will be clearly seen that only a very low accuracy is obtained. In reality most of the work must be said to be rather qualitative than quantitative.

For clinical purposes HOWELLS method was too complicated and a more simple method was described by HESS (1915, 1916) using recalcificated oxalated plasma as the source of thrombin and fibrinogen. Thus there is no incubation of a thrombin-antithrombin mixture and cannot be differentiated between antithrombic and antiprothrombic substances. The method has not been used since.

With the discovery of heparin by HOWELL and HOLT (1918) the problem was divided into two, namely the question about the antithrombin normally present in plasma and serum, and that of the antithrombin resulting from the addition of heparin. Only the first problem is to be discussed in this paper.

At this time antithrombin was known to be a substance which was more stable against heating than thrombin, as it was only destroyed at 65—70°. Its reaction with thrombin proceeds slowly at low temperatures but more rapidly at higher temperature. The nature of the substance and the inactivation process were still obscure, but it was known to be of a rather specific nature as serum was much more active than other substances tried, including proteins. It is destroyed by organic solvents.

In the following years only little was added to our knowledge about the normal antithrombin (see, f. i., WÖHLISCH (1929, 1940)). Some authors go as far as to deny the existence of a natural antithrombin existing in circulating blood (PICKERING and HEWITT (1921, 1922), PICKERING (1925)), but most of the experiments of the authors mentioned may just as well be explained through some known mechanism, e. g. by the presence of antithrombin. A thermostable antithrombin isolated from plasma by BARRATT (1929) may be due to traces of heparin, which also may be the case with all other thermostable antithrombic substances isolated from different tissues by various authors.

Interesting investigations, especially concerning the inability of HOWELLS antithrombin theory to explain the mechanism of blood coagulation were reported by J. BORDET (1920), GRATIA (1921) and P. BORDET (1929 a, b). But no definite differentiation between normal antithrombin and antithrombin from heparin or other sources (pepton plasma) was carried out in these works. The increase in the clotting time by addition of thrombin was as a rule used as a measure. Especially P. BORDET showed in some interesting experiments that the clotting time is increased not only by serum heated to 56° but also by serum heated to 70°, and he assumes therefore that the delayed coagulation in this case is not due to a specific antithrombin. He found that the reaction between antithrombin and thrombin only is completed in about 10 min. and that the amount of antithrombin is great in proportion to the amount of thrombin which can be formed from the same amount of serum. Chloroform inactivates antithrombin, but tricalcium phosphate does not adsorb it.

Based on his now definitely abandoned theory of "tissue fibrinogen" MILLS (1926) presented some highly improbable views concerning the nature of antithrombin. It seems possible that he may have worked with diluted heparin solutions.

MILLS (1926) and MILLS & KITZMILLER (1926) tried to determine antithrombin under certain pathological conditions, but they used methods even more inaccurate than HOWELL's. Later MILLS (1927) described an improved method in which purified thrombin and fibrinogen solutions were used, and which must be said to be better than HOWELL's method. One of the features of this method is the use of very active thrombin solutions.

It is only in recent years that any further steps towards a better understanding of the normal antithrombin have been made, and also of its relation to the antithrombin from heparin. Especially QUICK (1938) has made an important contribution, as he showed that the antithrombin is associated with the serum albumin and not with the serum globulin. Further he finds that an amount of plasma is clotted even by a very small fraction of that amount of thrombin which it is possible to inactivate by the antithrombin contained in the same amount of plasma, a fact which was also pointed out by MELLANBY (1909). He works with plasma as fibrinogen source and does not try to measure the amount of antithrombin quantitatively. Also WÖHLISCH and GRÜNING (1940) and WÖHLISCH and KÖHLER (1940) find that

serum albumin is most effective, and that its properties are concordant with the properties of the serum itself. QUICK (1938) is further of the opinion that it is the normal antithrombin which is increased in potency by addition of heparin, but this seems not to be true, according to experiments to be published later, cf. ASTRUP and DARLING (1941 b).

In a qualitative way an increase of antithrombin in obstructive jaundice was found by BARLIK (1933). This was later confirmed by DYCKERHOFF and MARX (1940) using a qualitative method which in principle is the same as the method used already by BORDET and GENGOU (1901). No incubation is employed and diluted magnesium sulphate plasma is used as fibrinogen source instead of avian plasma. Using the same unsatisfactory method, DYCKERHOFF and RUHL (1940) confirmed the results of BORDET and GENGOU (l. c.) as to the appearance of an antithrombic substance during immunization, but found that it is not due to the formation of an immune antithrombin but of an unspecific antithrombic substance which also is found after injection of foreign proteins. These questions have now been investigated in detail by VOLKERT (l. c.) using our method for the quantitative measurements.

JAQUES and MUSTARD (1940) incubate for 24 hours at 0° and find the antithrombin in the albumin fraction, but not in the crystalline serum albumin. According to their opinion, the slight activity of the antithrombin makes its measurement difficult.

Quantitative measurements on normal antithrombin have been attempted by WILSON (1940). He dilutes serum about 50 times and incubates with thrombin for 4 min. at 28°; and he corrects the result by multiplying with a factor found empirically. His method seems not to be based on rational principles.

Also HAWKINS and BRINKHOUS (1936) measure antithrombin by incubating with a thrombin solution of known strengh, but their method does not seem to be described in detail anywhere.

Our method therefore seems to be the first by which it is possible to measure antithrombin with any reasonable accuracy. This result has been achieved by taking the following points into consideration:

1) Only purified antithrombin-free reagents are used for the measurements.

2) A very active thrombin solution is used, because only in this case it is possible to say something about the presence of

any antithrombic substances. In a process as complicated as the clotting of blood the variation in the potency of a thrombin solution of low strength may be due to other influencing reactions than a variation of the antithrombin content.

3) Only a fraction of the thrombin present is neutralized by the addition of antithrombin, so that the remaining activity may be determined with as great an accuracy as the original activity.

4) The thrombin-antithrombin mixture is incubated at 37° until full saturation.

5) The amount of thrombin is kept constant and the amount of antithrombic material added is varied. This is contrary to HOWELL's method and gives the most uniform experimental conditions for the different samples, as in all cases only a fraction of the thrombin is inactivated.

6) For the sake of increasing the accuracy, several samples are incubated, and the clotting time of each is measured several times.

Using this method we have investigated the normal antithrombin from ox plasma. As is seen from the experimental part the properties found are in agreement with the experiences of earlier authors.

The amount of antithrombin in normal plasma and serum seems to be from about 100 to 150 A. T. U. per ml with a slight variation from species to species. According to our thrombin investigations, (ASTRUP and DARLING (1941 a)), about 70,000 T. U. are found in the activated solution of MELLANBY-fibrinogen from 10 L. of ox plasma, *i. e.* 7 T. U. per ml of plasma. It must be supposed that the yield is only a fraction of the total amount of thrombin, which can be formed from one ml of plasma, but nevertheless it is seen that the amount of thrombin is small in comparison with the amount of antithrombin in the same amount of plasma.

Summary.

1. A unit for antithrombin is defined as the amount of antithrombic substance which inactivates one unit of thrombin.

2. A method for the quantitative measurement of antithrombin in plasma and serum is described. Its relation to earlier unsatisfactory methods is discussed.

3. The normal antithrombin of ox plasma is investigated and its properties found in accordance with those described by other authors.

4. The amount of normal antithrombin in plasma and serum generally ranges from 100 to 150 antithrombin units per ml.

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On the Prothrombin Content in Milk.

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Prothrombin is chiefly found in blood plasma and only a few investigations have been carried out into the appearance of prothrombin outside the blood vessels. HOWELL (1915) has demonstrated prothrombin in the lymphatic plasma but none in the lymphatic cells. SMITH, WARNER and BRINKHOUS (1934) have not been able to demonstrate prothrombin in the lungs of rabbits, which have been perfused with saline. THORDARSON (1941) has shown the presence of small amounts of prothrombin in ascitic fluid, exudates and pathological spinal fluid, but could not demonstrate prothrombin in aqueous extracts of lung, liver, heart and breast musculature from rabbits. Milk has not previously been studied.

It was found that the content of prothrombin in colostrum from the cow is considerable, whereas the colostral secretion from women is poor in prothrombin. The following paper contains the results of prothrombin determinations in milk. It can be demonstrated that in the cow a hyperprothrombinemia occurs simultaneously with the appearance of considerable amounts of prothrombin in milk. The prothrombin content in colostrum is of no importance to the prothrombin concentration of the blood of the newborn calf.

Technique.

The prothrombin determinations in milk and blood plasma have been carried out according to THORDARSON's *method* (1941). By this procedure the prothrombin content is determined in relation to a standard

prothrombin solution, the prothrombin concentration of which has been arbitrarily put at 100 per cent. The determinations of prothrombin in cow's milk and cow's plasma have in this work been carried out in relation to a standard prothrombin solution which consisted of equal parts of fresh blood plasma from two normal, non-pregnant cows (171 and 207). This plasma can be considered as a suitable standard prothrombin solution for determination of prothrombin in cow's milk and blood plasma, because fresh samples of plasma mixtures from the two animals only deviate slightly from the one day old samples from the same animals, and a constant proportion is found between the content of prothrombin in the blood of normal, non-pregnant cows and the standard plasma solution. It is not possible to employ a human plasma as a standard prothrombin solution, as the relation between prothrombin concentration and clotting time for cow's plasma and human's plasma is different. The prothrombin content in the milk of women is determined in relation to a plasma from a normal person, the plasma prothrombin concentration of which has been determined to be 85 per cent prothrombin in relation to the standard of Thordarson for human beings. The accuracy of the method is 5 per cent. In case of determinations of prothrombin in milk with low prothrombin content the accuracy of the method is smaller, as the clotting times are long and do not agree very well.

The examined cows are from the neighbourhood of Aarhus. The blood samples from cows are drawn from the jugular vein. The blood samples and the milk samples have been determined the day on which they have been taken. The samples of woman's milk are from patients in the Lying-in Hospital for Jutland, Aarhus.

Results.

Prothrombin in cow's milk. The normal cow's milk, i. e. milk from cows not before 10 days after and at the latest three month before calving contains only negligible amounts of prothrombin, which appears from Table I.

Table I.

Cow No.	Milk prothrombin in per cent of prothrombin content of standard plasma
159	0.45
216	0.45
144	0.63
166	0.43
215	0.53
171	0.48
207	0.55
401	0.35
402	0.35
403	0.42
Average	0.46 %

In the months before and directly after calving one can, however, demonstrate considerable amounts of prothrombin in the milk, which appears clearly from Fig. 1, in which the results of the prothrombin determinations at different times about the calving are plotted. It follows from Fig. 1, that about 50 days before calving the prothrombin content of the cow's milk begins to increase.

The prothrombin appears at the time, where the milk secretion starts to decrease and the cow is going to be dry. At parturition the udder secretion contains about 20 per cent prothrombin, and after calving the prothrombin content decreases rapidly, and is below 1 per cent after 3 days. In all 44 milk prothrombin determinations were carried out on 20 different cows. Table II shows the decrease of prothrombin in the milk (colostrum) from a single cow.

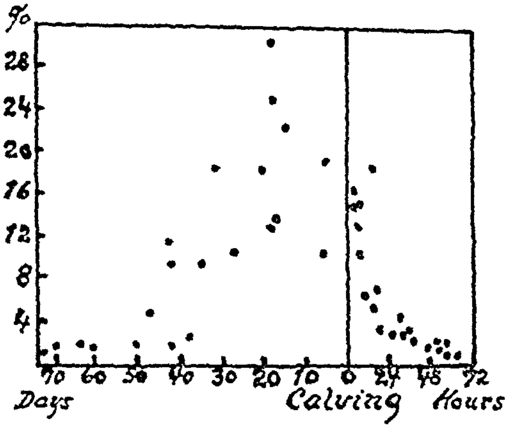

Fig. 1.

The ordinate gives the milk prothrombin in per cent of the prothrombin content of the standard plasma.

The asbcissa gives the days before and the hours after calving.

Table II.

Cow No. 224.

Hours after calving	Milk prothrombin in per cent of prothrombin content of standard plasma
2	15
6	15
10	11
18	6.8
26	5.8
30	2.0
42	2.4
52	1.5
60	1.5
66	0.9
78	0.9

THORDARSON (1941) and other investigators have in pregnant women demonstrated an increase of the prothrombin content of blood. It has therefore been natural to look for the same symptom in pregnant cows and particularly about the time of parturition, when the prothrombin content of milk is relatively high. For practical reasons only a few animals have been examined more then once. In all 46 blood samples have been drawn from 35 animals during pregnancy and after calving. The results of the prothrombin determinations in blood of cow's are plotted in *Fig. 2*.

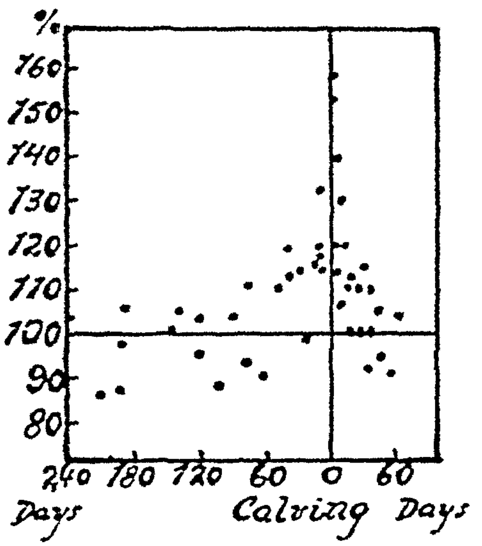

Fig. 2.

The ordinate gives the blood prothrombin in per cent of the prothrombin content of the standard plasma.

The abscissa gives the days before and after calving.

It appears from Fig. 2 that the values for the prothrombin per cent in blood of cows show a tendency to increase about two months before calving. The climax is about the time of calving and the values seem to be at a normal level about a month after calving.

Calves like newborn infants are born with a low prothrombin content in blood, and in a few days the prothrombin content increases to a normal level. It is generally maintained that the transitory hypothrombinemia in newborn infants is due to lack of vitamin K, and that the increase in prothrombin content of blood cannot appear until the child has received K-vitamin from outside with the natural nourishment or has produced the vitamin K itself by means of the bacteria present in the intestine. SMITH and LITTLE (1922, 1923) and ORCUTT and HOWE (1922) have shown that the colostrum of the cow contains certain antibodies, and that these antibodies are absorbed from the intestine of the calf. It has therefore been the object of our investigations to examine whether the prothrombin in colostrum like some antibodies is able to pass directly from colostrum to the blood of the newborn calf. The following experiment shows, however, that this is hardly the case. In April 1942 we got the opportunity to examine two

twin-calves immediately after birth. Calf No. 1 got the mother's colostrum milk in measured amounts and with known prothrombin contents. Calf No. 2 got ordinary fresh cow's milk. The prothrombin content of blood of the calves was tested during several hours after parturition. The details of the experiment were as follows:

Calf No. 1, ♀, born on April the 23rd at 10.30. Weight at birth 25.2 kg. Got on April the 23rd at 18.00 1.2 litre colostrum (prothrombin content: 13 %), on April the 24th at 8.00 1.2 litre colostrum (prothrombin content: 4 %). In all the colostrum given to calf No. 1 corresponds to 228 cc of the standard prothrombin solution (prothrombin content: 100 %).

Calf Nr 2. ♂, born on April the 23rd at 12.00. Weigth at birth 22.6 kg. At the same times as No. 1 it got fresh cow's milk in the same quantities (prothrombin below 0.5 per cent).

The prothrombin concentration in the blood of the mother of the calves on the day of calving was 159 per cent.

The results of the experiment appear from Table III. It appears that in the newborn calf no difference in the increase of the blood prothrombin concentration could be demonstrated whether the calf received colostrum rich in prothrombin or ordinary milk poor in prothrombin.

Table III.

Calf No. 1. Time in hours after birth	Plasma prothrombin per cent	Calf No. 2. Time in hours after birth	Plasma prothrombin per cent
0 hours 15 min.	78	1 hours 15 min.	72
2 » 30 »	72	3 » 15 »	68
4 » 30 »	68	5 » 25 »	72
6 » 30 »	74	7 » 15 »	72
8 » 30 »	70	9 » 15 »	83
10 » 30 »	80	11 » 30 »	74
12 » 45 »	78	14 » 45 »	75
16 » 0 »	78	18 » 15 »	88
19 » 30 »	88	21 » 15 »	95
22 » 30 »	94	24 » 15 »	88
25 » 30 »	88	28 » 15 »	95
29 » 30 »	86	31 » 45 »	97
32 » 30 »	100	35 » 15 »	97
36 » 30 »	95	45 » 0 »	105
46 » 55 »	112		

Woman's milk, also the colostral secretion, is poor in prothrombin as compared with cow's milk. This appears from Table IV, which gives the results of prothrombin determination on milk samples from a single patient. Other cases have given similar

results and Table IV thus demonstrate the facts sufficiently. There is no difference in the prothrombin content of milk taken in the beginning or at the end of one nursing.

Table IV.

Patient: M. S. Partus on June the first at 13.00.

Time in hours after birth	Milk prothrombin in per cent of the prothrombin in the standard plasma for human beings
22 hours 0 min.	1.6
24 » 30 »	2.0
27 » 20 »	1.4
30 » 30 »	1.4
36 » 30 »	3.2
42 » 30 »	1.8
45 » 30 »	0.2
48 » 30	0.2

10 other samples on the 3rd and 4th of June showed a prothrombin content below 0.2 %.

It should be mentioned that in regard to prothrombin content the dog's colostrum contains prothrombin of the same order of magnitude as the cow's colostrum. In dogs and rabbits we have not been able to demonstrate a sure increase of the blood prothrombin level during pregnancy.

Discussion.

The present investigation shows that ordinary cow's milk contains about 0.46 per cent prothrombin. About 50 days before calving when the cow is going to be dry the prothrombin concentration starts to increase and is about 20 per cent at the time of calving. In the first days after birth the calf receives no negligible amounts of prothrombin through the milk, but the prothrombin in the milk seems to play no part for the new-born calf. A hyperprothrombinemia can be demonstrated in the pregnant cow the last 50 days of pregnancy. This hyperprothrombinemia preserves the cow against a fall of plasma prothrombin owing to the loss of prothrombin through the mammary gland. It is not surprising that one should be able to demonstrate prothrombin in the cow's colostrum, as it is a well-known fact that the colostrum has a higher globulin content than ordinary milk. CROWTHER and

RAISTRICK (1916) have shown that lactoglobulin from colostrum is very closely allied to, and is probably identical with, serum globulin. SCHMITZ (1933) and others have demonstrated that prothrombin is found in the serum globulin faction. The colostrum from the dog contains prothrombin of the same order of magnitude as the cow's colostrum. Human milk contains below 0.25 per cent prothrombin. The colostrum from the woman does not contain more than 3.2 per cent. — The question whether the secretion of prothrombin in milk and the hyperprothrombinemia are brought about by the same impulse cannot be answered at present. Loss of prothrombin by bleeding does not seem to influence the prothrombin level of the blood. It should be mentioned that the hyperthrombinemia in pregnant women can be demonstrated from the third month of pregnancy, i. e. at a much earlier stage than in the pregnant cow. The average plasma prothrombin concentration in women at parturition is found to be 173 per cent with a standard deviation of 42, (SCHØNHEYDER and OLSEN, 1942). The average plasma prothrombin concentration in cows at the time of parturition is 134 per cent with a standard deviation of 17. It is to be supposed that the loss of prothrombin in women through the colostral secretion is much smaller than in the cow. It has not been possible to demonstrate a sure increase of the blood prothrombin level in pregnant dogs and rabbits.

Summary.

The milk prothrombin level was studied in cows and human beings. In the months before and directly after calving one can demonstrate considerable amounts of prothrombin in cow's milk. The woman colostrum also contains prothrombin but only in small amounts. At other times in the lactation period the cow's milk and human milk contain only negligible amounts of prothrombin.

The prothrombin content in the cow's colostrum is of no importance to the increase of the prothrombin in the blood of the new-born calf.

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Uber eine Depressorsubstanz, die sich im Serum in vitro bildet.

Von

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Eingereicht am 1. Juli 1942.

Bei der Untersuchung eines möglicherweise im Blut vorkommenden Histamins zeigte es sich, dass Serum, das einige Tage bei Körpertemperatur aufbewahrt worden war, eine stark blutdruckssenkende Wirkung bei der Katze ausübte. Da sich dieser Einfluss nicht auf einen der bekannten blutdrucksvermindernden Stoffe (Histamin, Acetylcholin, Adenosinverbindungen, Kallekrein) zurückführen liess, hielten wir es für angezeigt, diese Wirkung und denjenigen Stoff näher zu untersuchen, welcher dieselbe hervorruft. Die Entstehung eines solchen Stoffes hat für die Serumherstellung und die Aufbewahrung von Serumpräparaten natürlich grosse Bedeutung.

Wir arbeiteten in der Regel mit Pferdeserum, da man solches in verhältnismässig grossen Mengen erhalten kann. Rinder- und Menschenserum zeigten jedoch dasselbe Resultat. Das Blut wurde in sterilen Glaszylindern aufgefangen, und nach 24—48 Stunden wurde das Serum in sterile Kolben überführt, die dann im Thermostaten bei 37—40° C. zwei bis mehrere Tage lang aufbewahrt wurden. Wurde das Serum bei Zimmertemperatur gehalten, so blieb die blutdruckssenkende Wirkung aus, und zwar auch bei Gegenwart der Blutkörper in nicht defibriniertem Blut.

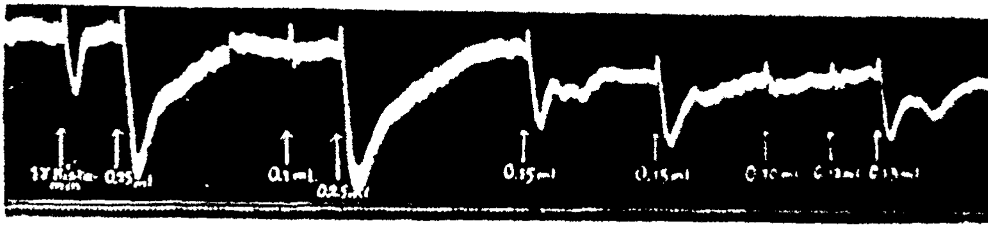
Bei der in Uretannarkose bei der Katze vorgenommenen intravenösen Injektion von 0.1—0.2 ml auf diese Weise behandelten Serums trat eine Blutdruckssenkung ein, die einer Wirkung von 1—5 γ Histaminhydrochlorid entsprach (Abbild. 1). Eine

Atropinisierung hob weder den Effekt auf noch verminderte sie ihn.

Isolierter überlebender Meerschweinchendarm wurde nicht beeinflusst.

Bei intravenöser Injektion von 2 ml stark wirksamen Serums bei einem nicht narkotisierten Kaninchen oder Meerschweinchen wurde keine klinisch nachweisbare Wirkung erzielt.

Auf Grund dieser Verhältnisse lassen sich, wie gesagt, Histamin, Acetylcholin, Adenosinverbindungen und Kallekrein als hier wirksame Stoffe ausschliessen.



Abbild. 1. Wirkung verschieden grosser Dosen aktiven Serums auf den Blutdruck bei der Katze in Uretannarkose. 0.12 ml Serum ohne Wirkung, 0.13 ml Blutdruckssenkung. Erste Injektion 1 γ Histaminhydrochlorid, in 0.25 ml physiol. Kochsalzlösung gelöst.

Die Wirkung ist jedoch von Histaminnatur und die Blutdruckssenkung beruht auf einer Dilatation der Kapillaren.

Bei näherer Untersuchung des Einflusses wechselnder Mengen auf den Blutdruck zeigte es sich, dass eine gewisse Dosis unwirksam war, während eine unbedeutende Steigerung der Menge eine kräftige Blutdruckssenkung verursachte. Es bedarf mit anderen Worten einer scharf markierten Mindestmenge, um eine Blutdruckssenkung zu erhalten (Abbild. 1).

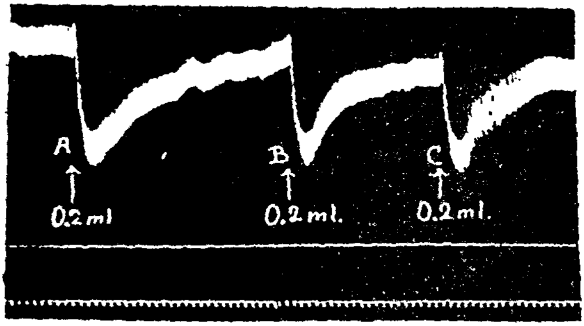
Zwecks näherer chemischer Kennzeichnung der wirksamen Substanz wurde aktives Serum chemischen Behandlungen unterworfen und die verschiedenen Fraktionen bei der Katze und zur Kontrolle auch am isolierten Meerschweinchendarm geprüft.

Nach der Eiweissfällung waren die Filtrate stark aktiv. Bei den verschiedenen Behandlungen, und zwar vor allem bei der Fällung des Eiweisses aus dem Serum, traten jedoch grosse Verluste ein, die zum grossen Teil sicher auf einer Adsorption der Eiweissfällungen beruhte. Im übrigen scheint die wirksame Substanz bei Zimmertemperatur verhältnismässig stabil zu sein und den Einfluss verschiedener Reagenzien zu ertragen. Nach der

Aufbewahrung in wässeriger Lösung nimmt aber die Aktivität bei gereinigten Präparaten nach einiger Zeit ab.

Die Eiweissfällung geschah mittels Koagulierung bei 80° C. nach schwacher Ansäuerung mit Essigsäure oder mit Alkohol, Ammoniumsulfat oder Trichloressigsäure.

Das Filtrat war nach der Koagulierung in der Wärme ebenso stark aktiv wie das Ausgangsmaterial, aber wegen des Umfanges der Eiweissfällungen wurde nur eine geringe Menge Filtrat erhalten. Die Fällung mit Alkohol geschah in der Weise, dass zunächst mit dem gleichen Volumen 95proz. Alkohols oder einer



Abbild. 2. Katze in Uretannarkose. A = Serum. B = Filtrat von Serum nach Fällung mit dem gleichen Volumen einer Mischung von Alkohol und Äther. Filtrat eingedampft im Vakuum und in physiol. Kochsalzlösung gelöst. C = Filtrat von B nach Fällung mit dem dreifachen Volumen von Alkohol + Äther sowie Behandlung wie oben angegeben.

Mischung von 3 Teilen Alkohol und 1 Teil Äther gefällt wurde: Nach dem Eindampfen des Filtrates im Vakuum bei 55° auf den zehnten Teil des ursprünglichen Volumens wurde eine neue Fällung mit dem dreifachen Volumen Alkohol vorgenommen. Nach der Verdunstung des Alkohols im Vakuum wurde ein stark aktives Präparat erhalten, obgleich grosse Verluste teils durch Absorption der Fällungen, teils beim Eindampfen entstanden (Abbild. 2).

Beim Gebrauch grösserer Mengen von Ammoniumsulfat als eiweissfällenden Mittels ergaben sich nach der Dialyse zur Beseitigung der Ammoniumsalze wirkungslose Filtrate. Wurde die Ammoniumsulfatmenge vermindert, so waren die Filtrate wirksam, aber das Eiweiss wurde nicht vollständig gefällt.

Nach der Eiweissfällung mittels Trichloressigsäure wurde das Filtrat mit Äther bzw. Petroleumäther zur Beseitigung des Fällungsreagenzes ausgeschüttelt. Auch hierbei wurden aktive Fil-

trate erhalten, aber weitere Substanz liess sich aus der Eiweissfällung durch deren Ausschüttelung mit geeigneten Lösungsmitteln erzielen.

Diese Versuche zeigen jedoch, dass die Substanz nicht aus Eiweiss oder hochmolekularen Spaltprodukten von Eiweiss besteht.

Bei Reinigungsversuchen durch Adsorption zeigte es sich, dass Kaolin die Substanz nicht adsorbierte, dagegen Aluminiumhydroxyd. Es war aber nicht möglich, die Substanz auch in Versuchen mit verschiedenen pH-Werten wieder zu eluieren.

Die wirksame Substanz war bei neutraler oder schwach alkalischer Reaktion in Wasser löslich. Bei Ansäuerung bis zu Blaufärbung mit Kongopapier einer eiweissfreien Lösung wurde die Substanz gefällt. Dieselbe war ferner in 95proz. Alkohol und Äther löslich.

Bei Versuchen zur Reinigung der Substanz durch Fällung mit Bleiacetat oder Silbernitrat bei verschiedener Wasserstoffionenkonzentration, wodurch eine Fraktionierung von Aminen bewirkt wurde, trat keine Scheidung derselben ein, sondern Aktivität wurde in sämtlichen Fraktionen erhalten.

Die nach Ansäuerung erhaltene Fällung liess sich wieder in Wasser bei schwach alkalischer Reaktion lösen, wobei eine etwas opalisierende Lösung erhalten wurde. Wurde die genannte Fällung mit 95proz. Alkohol oder Äther extrahiert, so ergaben sich nach Eindampfung dieser Lösungsmittel und Lösung des Rückstandes in schwach alkalischer wässeriger Lösung aktive Präparate.

Ein solches, durch wiederholte Fällungen gereinigtes Präparat enthielt ausser anorganischen Salzen auch Phosphatide. Durch Phosphorbestimmung liess sich der Phosphatidgehalt im organischen Teile des Präparats auf ungefähr 50 Prozent, als Lecithin berechnet, feststellen. Die Substanz kann somit eventuell Phosphatidnatur besitzen, doch liess sich dieses noch nicht mit Sicherheit beweisen.

Bei Dialyseversuchen durch Kollodiummembran passierten in der Zeit von 4 Stunden keine wahrnehmbaren Mengen wirksamer Substanz. Wurde die Dialysezeit auf 24 Stunden erstreckt, so liess sich die Substanz auch ausserhalb der Dialysierhülse nachweisen.

Nach einer 30 Min. langen Erwärmung aktiven Serums auf 60—65° C. nach schwacher Ansäuerung mit Salzsäure war die

Aktivität unverändert. Bei alkalischer Reaktion wurde dagegen der wirksame Bestandteil bei obengenannter Temperatur und Zeit vollständig zerstört. Ein eiweissfreies gereinigtes Präparat in neutraler Lösung wurde nach 2 Min. langem Kochen oder nach 30 Min. langer Erhitzung im kochenden Wasserbad inaktiviert. In 1 n HCl wurde die gereinigte Substanz nach 10 Min. langer Erwärmung im kochenden Wasserbad völlig inaktiviert.

Zwischen der von uns beschriebenen Substanz und den Serotoxinen liegt eine gewisse Übereinstimmung vor. Letztere zeigen jedoch auch gewisse Abweichungen. Sie entstehen bei der Berührung mit gewissen Kontaktsubstanzen, wie gekochtem Eiweiss, Bakterien, Pepton, Agar-Agar, Stärke, gewissen anorganischen Verbindungen u. a. Dagegen bilden sie sich nicht in frischen Seren bei Erwärmung auf 37° C. (JOBLING und PETERSEN, LÖWIT, DOLD). Die Serotoxine verursachen bei intravenöser Injektion bei Meerschweinchen eine Schockwirkung. Sie werden unverändert von Alkohol gefällt (FRIEDBERGER und KONITZER).

Grössere Übereinstimmung zeigt eine Substanz, deren Wirkung von ZIPF und WAGENFELD beschrieben wurde. Frisch defibriniertes art- oder körpereigenes Blut bewirkt in einer Menge von 1—5 ml bei Kaninchen bei intravenöser Injektion eine mehr oder weniger starke Herabsetzung des Blutdrucks. Die Wirkung ist von Histaminnatur. Sie bleibt nach der Ausfällung des Eiweisses bestehen. Bei längerer Erhitzung über 70° C. wird sie zerstört.

Diese Substanz wurde pharmakologisch von HAAKE geprüft, der fand, dass sie tonusherabsetzend und bewegungshemmend auf überlebenden Kaninchendünndarm sowie tonussteigernd und erregend auf überlebenden Meerschweinchenuterus wirkte. Es handelt sich hier jedoch um defibriniertes Blut, während unsere Substanz im Serum gebildet wurde.

Zusammenfassung.

Bewahrt man steriles Serum von Mensch, Pferd oder Rind einen bis mehrere Tage bei 38° C. auf, so bildet sich eine Substanz, die bei intravenöser Injektion bei der Katze oder beim Kaninchen eine stark blutdruckssenkende Wirkung besitzt. Diese Wirkung ist von Histaminnatur, die Substanz ist aber mit diesem Stoff oder anderen bekannten Depressorsubstanzen nicht iden-

tisch. Zur Herbeiführung einer Blutdruckssenkung ist eine gewisse Mindestmenge der betreffenden Substanz erforderlich.

Überlebender Meerschweinchendarm wird nicht beeinflusst.

Die Wirkung bleibt bestehen, wenn das Eiweiss aus dem Serum ausgefällt wird.

Die Substanz ist ausser in Wasser auch in Alkohol und Äther löslich.

Aktives Serum erträgt eine 30 Min. lange Erhitzung auf 60—65° C., ohne dass die Wirkung beeinträchtigt wird. Gereinigte Präparate sind dagegen temperaturempfindlich.

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Über das Austreten von Kalium aus den roten Blutkörperchen im Reservoirblut der Milz.

Von

NILS BRAGE NORDLANDER.

(Eingereicht am 3. Juli 1942.)

Im Jahre 1921 lenkte BARCROFT das Interesse auf die Milz durch das Studium ihrer Reservoirfunktion. Er fasste diese hauptsächlich als eine rein quantitative Regelung der Menge der roten Blutkörperchen im zirkulierenden Blut auf. So zeigte er, dass das Reservoirblut der Milz sehr reich an roten Blutkörperchen ist, die bei Bedarf in den allgemeinen Kreislauf ausgeschüttet werden. Die Mechanik der Reservoirfunktion war lange dunkel, bis KNISLEY 1936 durch Untersuchungen an lebenden Tieren zeigte, was wohl das Wesentliche in den eigenartigen Kreislaufverhältnissen der Milz ist. Nach KNISLEY sind die Wände der Venensinus Filter, welche die roten Blutkörperchen zurückhalten, das Plasma aber passieren lassen. Diese Separation kommt folgendermassen zustande. Ab und zu wird der Kreislauf infolge Kontraktion eines Sphinkters an dem venösen Ende des Sinus unterbrochen, wobei der Sinus sich mit Blutkörperchen füllt, während das Plasma durch die Sinuswand hinausdringt. Wenn der Sinus sich mit Blutkörperchen gefüllt hat, schliesst sich ein Sphinkter auch an dem arteriellen Ende, und die Blutkörperchen werden in »deplasmatisiertem» Zustand bis zu 10 Stunden aufgespeichert. Durch Öffnen der Sphinkter und Kontraktion des Sinus werden sie alsdann in den Kreislauf ausgeschüttet.

Schon 1921 stellte FÅHRAEUS fest, dass, wenn man Blut in vitro bei Körpertemperatur einige Stunden aufbewahrt, die roten Blutkörperchen sphärisch werden und ihre Neigung zu Geldrollen-

bildung sowie ihre Sedimentierungsgeschwindigkeit abnehmen. Der Prozess, der diese Blutumwandlung verursacht, ist »der Stabilisierungsprozess« genannt worden und das veränderte Blut »stabilisiertes Blut«. Die Stabilisierung, die veränderte Eigenschaften sowohl der roten Blutkörperchen als auch des Plasmas in sich schliesst, wurde anfangs als eine Folge des Stillstandes des Blutes gedeutet, da sie nicht in geschüttelten Blutproben eintritt. Es ist jedoch nicht der Stillstand an sich, der die Bedingungen für die Entstehung der obengenannten Stabilisierung darstellt, sondern der Stillstand bildet nur die Voraussetzung für die Sedimentierung der roten Blutkörperchen, wodurch also eine Trennung von Erythrozyten und Plasma während der Erwärmung zustande kommt. Wie BERGENHEM & FÅHRAEUS gezeigt haben, bestehen die veränderten Eigenschaften des Plasmas wahrscheinlich hauptsächlich in einer Bildung von Lysolezithin, während die nähere Natur der Erythrozytenveränderung immer noch unbekannt ist. Die genannten Verfasser nahmen an, dass die Veränderungen, die das Blut bei der Trennung von Blutkörperchen und Plasma in vitro erfährt, wahrscheinlich auch in der Milz stattfinden, wo wenigstens betreffs der Blutkörperchen dieselbe Voraussetzung vorliegt wie bei den In-vitro-Versuchen, dass heisst die roten Blutkörperchen werden während einer Zeit, die in Stunden gemessen wird, in den Venensinus bei Körpertemperatur deplasmatisiert gehalten. Es liegen tatsächlich Beobachtungen aus der Mitte des vorigen Jahrhunderts über die besonderen Eigenschaften des Milzvenenblutes vor, und zwar, dass seine roten Blutkörperchen mehr sphärisch sind und verminderte Geldrollenbildung, somit auch geringere Senkungsgeschwindigkeit zeigen als die roten Blutkörperchen in sonstigen Teilen des Kreislaufes. Das Blut in der Milz scheint also eine ähnliche Veränderung durchzumachen wie das in vitro stabilisierte Blut.

Die Frage, die ich mir hier gestellt habe, ist, ob noch andere Sondereigenschaften des Milzreservoirblutes auf der Isolierung der Erythrozyten durch das Abfiltrieren des Plasmas beruhen können.

LUDÁNY und Mitarbeiter haben in einer Reihe von Untersuchungen mehrere Eigenschaften festgestellt, durch die sich das Reservoirblut der Milz von peripherem Venenblut unterscheidet. So fanden sie, dass der Kaliumgehalt im Reservoirplasma der Milz bei Hunden im Vergleich mit dem peripheren Blut um etwa 200 % vermehrt war. Die Erythrozyten sind bekanntlich reicher an Kalium als das Plasma, und die genannten Autoren nahmen als

Erklärung des erhöhten Kaliumspiegels im Plasma eine in der Milz eingetretene Hämolyse an.

Bei eigenen Versuchen habe ich bei Katzen eine Vermehrung um beinahe 100 % feststellen können.

Die Technik bei diesen Tierversuchen scheint vielleicht eine bessere Möglichkeit zu bieten, die Blutproben aus der Milz physiologisch zu erhalten als diejenige, die die genannten Verfasser benutzt haben (narkotisierte Tiere), weshalb ein typisches Beispiel aus dem Versuchsprotokoll wiedergegeben sei.

Eine 2.25 kg schwere Katze in Morphium-Numal-Narkose. Schnitt in der Mittellinie und Vorwälzen der Milz aus dem Bauch, wo man sie mit Kompressen umgab, die in warme, physiologische Kochsalzlösung getaucht worden waren. Diathermieschnitt durch das Oment und sorgfältiges Freipräparieren der Vena lienalis auf einer 1 cm langen Strecke. Durch einen kleinen Schnitt in die Vene wurde nach jeder Seite eine kleine gerade Glaskanüle eingeführt. Diese Kanülen waren durch einen 10 cm langen Gummischlauch miteinander verbunden. Der Schlauch und die Kanülen waren im Voraus zusammengebunden und inwändig mit einer dünnen Haut von einer 2 %-igen Heparinlösung in einer 5 %-igen Gelatinelösung überzogen worden. Diese Lösung wurde einige Male warm durch den Schlauch gespült, um nachher dort zu trocknen, wonach die Prozedur wiederholt wurde. Dann wurde der Schlauch in Trockenluft in einer Röhre im Ölbad während einer Stunde bis auf 130° erwärmt, wobei er sterilisiert wurde und die Gelatine sich in eine wasserfestere Substanz verwandelte. Der so präparierte Schlauch mit seinen Kanülen wurde mit Ligaturen in der Milzvene befestigt. Die Milz wurde in den Bauch zurückgelegt, der unter minutiösem Blutstillen geschlossen wurde. Eine Schleife des Schlauches liess man bei der Vernähung ausserhalb des Bauches zurück. Die ganze Operation fand unter sterilen Massnahmen statt.

Nach 4 Stunden wurde durch den Schlauch eine intravenöse Injektion von 2 mg Heparin je kg Körpergewicht gegeben, und diese Injektion wurde anfangs jede zweite Stunde, später mit wachsendem Intervall wiederholt. In dieser Weise war es möglich, die Gerinnungszeit des Blutes über 10 Minuten zu halten (nach MORAWITZ gemessen, aber von dem Augenblicke an gerechnet, wo das erste kleine Gerinnsel aus der Probe herausgefischt werden konnte). Die Zirkulation im Schlauch war die ganze Zeit gut, die Katze erwachte allmählich, wurde lebhafter, frass und trank.

18 Stunden nach der Operation wurde dem Schlauch eine Probe entnommen, wonach 0.2 ccm 0.1%-ige Adrenalinlösung injiziert wurden, und nach 1/2 Minute wurde noch eine Blutprobe entnommen. Die Proben wurden auf Senkungsreaktion, Hämatokritenwert und Kaliumgehalt im Plasma untersucht.

	Senkungsreaktion	Hämatokritenwert	Kaliumgehalt
Probe 1	13 mm/Stunde	33.8 % Erythrozyten	16.8 mg %
Probe 2	1.5 »	45.2 » »	27.9 » »

Sowohl der Hämatokritenwert als auch die stabilisierte Senkungsreaktion beweisen, dass Probe 2 Reservoirblut darstellte, während Probe 1 nur Passageblut war. Der Unterschied im Kaliumgehalt ist augenfällig.

Diese Metodik ist hauptsächlich von Dr J. Mellgren zu anderem Zwecke ausgearbeitet worden und ich schulde ihm deshalb grossen Dank.

Die vorliegende Untersuchung will experimentell prüfen, ob der gesteigerte Gehalt an Kalium im Reservoirblut der Milz eine direkte Folge der Separation der Erythrozyten vom Plasma in den Sinus der Milz sein kann, ob also auch bei Separation in vitro Kalium aus den Erythrozyten austritt. Die Versuche sind an Katzen-, Pferde- und Menschenblut ausgeführt worden. Die Gerinnung des Blutes wurde durch Defibrinieren oder durch Zusatz von Zitrat oder Heparin verhindert.

Eine Reihe von Proben wurde zentrifugiert (2 000 Umdrehungen je Minute während 20 Minuten) und in einen Thermostaten von 40° gesetzt. Die Blutkörperchen lagen also in diesen Röhrchen auf dem Boden zusammengepackt mit einer klaren Plasmaschicht darüber. Eine andere Reihe mit ebenso vielen Röhrchen wurde in demselben Thermostaten in stetiger Rotation gehalten. Mit geeigneten Intervallen wurde ein Röhrchen aus jeder Reihe in einen Kühlraum (4° C) gestellt. Als sämtliche Röhrchen im Kühlraum waren, wurde der Inhalt jeder Röhrchen durch Umdrehungen während einer Minute gemischt und die Senkungsreaktion untersucht. Dann wurden sämtliche Röhrchen zentrifugiert, 1 ccm Plasma abpipettiert und auf seinen Kaliumgehalt untersucht. Proben mit geringstem Zeichen von Hämolyse wurden weggeworfen. Ich hatte die grosse Vergünstigung, die Kaliumanalysen spektrographisch am Pflanzenphysiologischen Institut, Ultuna, unter der Oberaufsicht und mit gütiger Unterstützung der Herren Prof. Lundegårdh und Dr Philipsson ausführen zu dürfen, wofür ich auch hier meinen warmen Dank sagen möchte.

Die Hauptergebnisse gehen aus den beistehenden Diagrammen hervor. Die dicken Linien bezeichnen die Sedimentierung der roten Blutkörperchen, die dünnen den Gehalt an Kalium im Plasma. Die ausgezogenen Linien bedeuten stabilisiertes Blut, die gestrichelten Linien Blut, das durch ununterbrochene Rotation an der Stabilisierung gehindert wurde. Abb. 1 zeigt, dass nach einer 9-stündigen Separation der Erythrozyten vom Plasma in defibriniertem Pferdeblut so viel Kalium ausgetreten ist, dass der Kaliumgehalt des Plasmas um 101 % gestiegen ist. In der

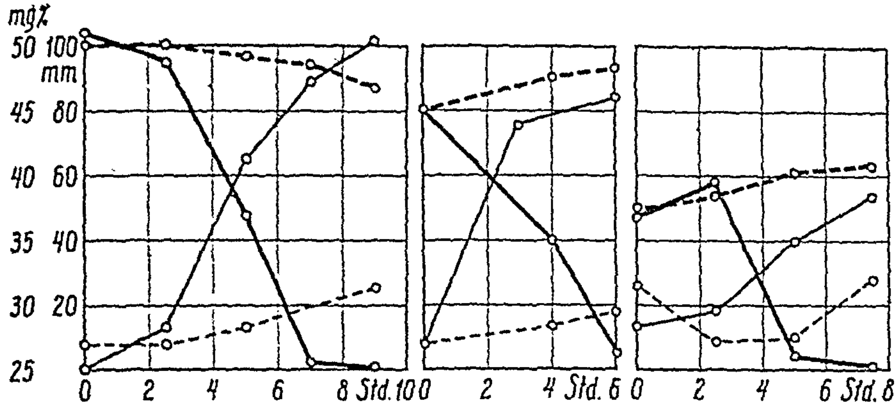

Abb. 1. Abb. 2. Abb. 3.

gleichzeitig erwärmten, aber die ganze Zeit rotierenden Probe, die also nicht stabilisiert ist, ist der Kaliumgehalt nur um $16^1/_2$ % gestiegen. Abb. 2 zeigt dieselben Verhältnisse bei Zitratblut vom Pferd. Die Steigerung des Kaliumgehalts beträgt hier nach 6-stündiger Stabilisierung 73 %, während die nicht stabilisierte Probe eine Steigerung um $9^1/_2$ % zeigt. Abb. 3 ist mit Heparinblut vom Menschen erhalten. Nach $7^1/_2$-stündiger Stabilisierung ist der Kaliumgehalt um 34 % gestiegen, in der nicht stabilisierten Probe nur um $1^1/_2$ %.

Die Senkungsreaktion zeigt in sämtlichen Kurven eine starke Stabilisierung, und die Blutkörperchen sinken am Ende der Versuche nur wenige Millimeter. Aus den Zwischenwerten geht deutlich hervor, dass sich die Durchlässigkeit der Blutkörperchenmembranen für Kalium und die Stabilisierung der Senkungsreaktion parallel entwickeln.

Das Hauptergebnis dieser Untersuchungen von Pferde-, Menschen- und Katzenblut ist, dass eine Separation der roten Blutkörperchen vom Plasma (bzw. vom Serum in defibriniertem Blut) bei Körpertemperatur in vitro einen Austritt von Kalium aus den roten Blutkörperchen hervorruft. Es hat sich also die bemerkenswerte Tatsache herausgestellt, dass eine *Separation* der beiden Hauptbestandteile des Blutes Voraussetzung eines Austausches zwischen ihnen bei einer nachfolgenden Vereinigung bedeutet. Aus angestellten Versuchen geht hervor, dass dieser Austausch sofort in vollem Ausmasse stattfindet, wenn Blutkörperchen und Plasma nach der Wärmebehandlung wieder gemischt werden. Mehrstündige Umdrehungen dieser Mischung bewirkt keinen gesteigerten Austritt von Kalium aus den roten Blutkörperchen.

Der oben beschriebene In-vitro-Versuch könnte als ein Modell für das Verhalten der roten Blutkörperchen in der Milz dienen: bei den In-vitro-Versuchen erfolgt die Trennung vom Plasma durch Zentrifugieren, in den Venensinus der Milz durch Filtrieren.

Die Freimachung einer gewissen Menge Kalium aus den roten Blutkörperchen kann auf zwei Arten stattfinden:

1) Totaler Zerfall einer Anzahl roter Blutkörperchen. Die Voraussetzung der grossen Steigerung des Kaliumgehalts des Plasmas wäre dann eine beträchtliche und zweifellos augenfällige Hämolyse, die aber niemals eingetreten ist.

2) Gesteigerte Durchlässigkeit sämtlicher roter Blutkörperchen für Kalium — ohne gleichzeitigen Zerfall — die durch eine Veränderung der Oberfläche eintreten müsste. Dass eine Umwandlung der Oberfläche in den bei Körpertemperatur gehaltenen, deplasmatisierten roten Blutkörperchen tatsächlich stattfindet, geht daraus hervor, dass, wie oben angedeutet, diese sphärisch werden und ihre Aggregabilität herabgesetzt wird, Veränderungen, die von Bergenhem und Fåhraeus als ein Vorstadium der Hämolyse gedeutet wurden. Hierfür sprechen auch Beobachtungen von Orahovats, wonach die roten Blutkörperchen des Milzvenenblutes gegen hypotonen Salzlösungen weniger resistent sind.

Die hier vertretene Auffassung, dass die Milz für den Stoffwechsel des Kaliums im Organismus von besonderer Bedeutung ist, weil in ihr Kalium aus den roten Blutkörperchen ins Blutplasma übergeht, kann vielleicht an interessante Beobachtungen von Henriques und Ørskov angeknüpft werden. Diese Forscher fanden, dass bei Anämie, die sie durch Aderlass oder Injektion von Phenylhydrazin bei Kaninchen und Hunden hervorriefen, der Gehalt der roten Blutkörperchen an Kalium erheblich stieg. Diese Tatsache wurde in der Weise gedeutet, dass die jungen Blutkörperchen, die bei diesen Zuständen in reicher Menge in die Blutbahn ausströmen, beträchtlich kaliumreicher sind als die alten Blutkörperchen und dadurch den übrigen Geweben des Körpers in bezug auf Kaliumgehalt näher stehen. Die Verfasser ziehen die Folgerung, dass das Altern der roten Blutkörperchen mit einem Verlust von Kalium verbunden ist. Meiner Meinung nach wird diese Veränderung durch wiederholte Milzpassagen hervorgerufen.

Zusammenfassung.

1) In Plasma von Reservoirblut der Katzenmilz ist der Kaliumgehalt im Verhältnis zum peripheren Blut um beinahe 100 % gesteigert.

2) Bei Erwärmung von roten Blutkörperchen und Plasma (bzw. Serum) von Katzen-, Pferde- und Menschenblut, das in vitro durch Zentrifugieren separiert worden ist, geben die roten Blutköperchen Kalium ab.

3) Es wird die Theorie aufgestellt, dass der gesteigerte Kaliumgehalt des Reservoirplasmas der Milz eine Folge der Separation der roten Blutkörperchen vom Plasma in den Venensinus bei der Reservoirfunktion der Milz ist.

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The Specific Dynamic Action of Amino Acids and Ammonia Salts.

By

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The expression "specific dynamic action" was introduced by RUBNER (1902) as a term denoting the stimulating action of foodstuffs on metabolism. The actual phenomenon that intake of food increases the basal metabolism was observed as long ago as by LAVOISIER. In the century and a half since LAVOISIER made this observation it has been the subject of innumerable investigations with the object of elucidating the fundamental cause of the increase of energy output induced by food absorption. But in spite of all the labour expended on experiments of this rather delimited problem, complete clarity has not yet been obtained. It is true that it has been possible to discard certain theories as being unsatisfactory, but a large number may still be said to be under discussion. Nevertheless, there seems to be general agreement that the proteins or the products of protein digestion — the amino acids — have a much greater effect on the basal metabolism than the nitrogen-free foodstuffs. It may also be said that most authors agree that the action of the amino acids is fundamentally different to that of the nitrogen-free substances. That the action of amino acids is not associated with the amino acids as such, but with their transformation in the organism is likewise recognized by the majority of those who have occupied themselves with the problem.

Without going into details, the theories concerning the action of the amino acids may be said to fall within two groups. The

action is ascribed either to the nitrogen-free part of the molecule and its breakdown or to the amino group and its further transformation into urea.

In 1916 GRAFE published experiments showing that ammonium salts possess a specific dynamic action. This observation did not earn general recognition, however, to a great extent due no doubt to the fact that GRAHAM LUSK in collaboration with ATKINSON (1918) was unable to substantiate it. LUSK is probably the scientist who has devoted most effort to the investigation of the specific dynamic action of foodstuffs. Still, his experiments on the influence of ammoniumsalts on metabolism are few and unconvincing.

In 1931 I published a series of experiments on the specific dynamic action, performed on dogs. These experiments included *a comparison of the action of amino acids and ammonium salts.* The ingestion of the same quantity of nitrogen in the form of alanine and ammonium lactate gave the same increase in the consumption of oxygen, whereas the ingestion of sodium lactate was without evident effect. Ammonium chloride also turned out to have the same action per gram of ingested nitrogen as the amino acids alanine and glycine. From these investigations I drew the conclusion that the specific dynamic action of proteins is associated with the amino group, either because the ammonia freed by deamination acts as a metabolism-increasing irritant, or because the synthetization of the ammonia to urea represents work and thus involves a consumption of energy. Two years ago (1940) OBERDISSE published his investigations on the specific dynamic action of the proteins, comprising inter alia experiments on the influence of ammonium salts on the oxygen consumption of the isolated artificially perfused liver. In these investigations OBERDISSE observed no action of ammonium salts. At the time when I became aware of these investigations I was engaged on experiments with artificially perfused isolated livers, and I therefore resumed my earlier studies of the specific dynamic action of amino acids and ammonium salts, employing a technique similar to that used by OBERDISSE.

Technique.

The experiments were performed on cat livers perfused via the portal vein by the technique usually employed in this institute (cf.

LUNDSGAARD, NIELSEN and ØRSKOV 1936). The cat blood used for perfusion was moderately diluted with Ringer solution, so that the oxygen capacity lay at about 12—13 volumes per cent. The weight of the livers was between 60 and 100 g., with an average weight of about 80 g. The minute volume, which is very constant within the individual experiment, varied from one experiment to an other from 50 to 70 c. c. according to the size of the liver.

As the aim of the experiments was an evaluation of the total extra consumption of oxygen after the addition of amino acids and ammonium salts to the perfusion blood, the application of a method of continuous recording of the oxygen consumption was of great importance. For this purpose I introduced a photo-electric recording of the oxygen content of the venous blood. The technique for this was the most simple imaginable. The venous blood from the liver passes a cell inserted between a constant light source and a photo-cell. The photocurrent is recorded by a galvanometer whose sensitivity is adjusted so that the changes in the oxygen content of the venous blood generally occuring in my experiments give a deflection of 5 to 7 cm. on the scale.

If in a coordinate system with a logarithmic division of the ordinate the galvanometer readings are plotted along the ordinate, and the blood oxygen content in volumes per cent along the abscissa, we obtain straight line curves with a constant incline independent of the haemoglobin concentration. On the other hand, the position of the curve in the coordinate system is determined by the haemoglobin concentration. A considerable number of determinations of the oxygen content of the venous blood according to VAN SLYKE were made in some preliminary experiments for the purpose of determining the inclination of the curve by comparing the results with the simultaneous galvanometer readings. A considerable variation of the oxygen content in the venous blood was obtained partly directly, in that the oxygen consumption of the isolated liver always falls considerably in the first 30—60 minutes after commencing artificial perfusion (see later), partly by altering the amount of oxygen taken out of each 100 c. c. of blood by ligaturing smaller or larger parts of the liver while keeping the minute volume constant. The inclination of the standardization curve having thus been established with sufficient accuracy, it was only necessary in the actual experiments to make a few VAN SLYKE determinations of the oxygen content in the venous blood. In theory a single determination should suffice to establish the position of the curve in the coordinate system; actually, five or six were made in order to find the position with greater accuracy and also for the purpose of checking the photo-electric measurements of the oxygen content. Changes in the osmotic concentration of the plasma, which cause the corpuscles to shrink or swell, compromise the photoelectric determinations, as well as changes in the haemoglobin concentration within the experimental period lead to errors. It was possible to provide almost complete compensation for the latter errors by calculating the oxygen consumption from the difference between

the photo-electrically determined oxygen content in the venous blood and the oxygen content of the arterial blood at the *beginning* of the experiment, determined by the VAN SLYKE method. A moderate increase in concentration of the blood has the effect that in the latter part of the experiment the oxygen content of the venous blood is read somewhat too low; but if at the same time we disregard the simultaneous increase of the oxygen content in the arterial blood, the error is compensated. There is no theoretical foundation for such a procedure, of course, but experience has shown that in practice it reduces greatly the errors due to a change in concentration of the blood. In experiments of about two hours' duration the increase in the oxygen capacity of the blood usually amounts to less than 0.5 vol. per cent.

In the experiments to be described in this article the additions made to the blood were glycine, alanine, ammonium lactate and ammonium carbonate. In most experiments the quantities correspond to 56 mg.N. dissolved in 5 c. c. water. The additions were made in a single dose by pouring the solution down into the blood reservoir of the oxygenator. This caused no osmotic changes capable of disturbing the photoelectric determination of the oxygen content in the venous blood.

The usual procedure was as follows: after commencing artificial perfusion the oxygen consumption of the liver was observed until it became constant; with great regularity this occurs in the course of about an hour. The great advantages secured by continuous photoelectric recording of the oxygen content in the venous blood become manifest by the fact that it is possible to determine with certainty when the oxygen consumption has reached a constant level. Furthermore, it is possible to draw samples of venous blood for oxygen determination after VAN SLYKE at such intervals that one obtains the greatest possible variation between the oxygen content in the samples, this being of importance for the accuracy with which the "standardization curve" can be laid. When the oxygen consumption of the liver has become constant one adds the substance the effect of which is to be tested and the oxygen consumption is again traced until it once more becomes constant, as it will usually do almost at the same level as before the addition. The galvanometer recordings are not photographed but read off at intervals of five minutes, except during a rapid increase in the oxygen consumption, when the intervals are of one or two minutes.

The oxygen content of the arterial blood was determined only together with the first and the last sample of the venous blood. The oxygen consumption was calculated in two ways: from the minute volume and the difference between the photo-electrically determined oxygen content in the venous blood and the first arterial blood sample (cf. above), and also from the VAN SLYKE determinations, the amount of oxygen taken out of each 100 c. c. of blood in the first half of the experiment being calculated with the oxygen content of the first arterial blood sample as a basis and in the latter half with the oxygen content of the last arterial blood sample.

The variations of the oxygen consumption are plotted in the form of curves from the photo-electric determinations. The total extra consumption of oxygen after the addition of the various substances is calculated from the area of the curve above a straight line connecting the curve level before and after addition.

The Experiments.

In a series of experiments a comparison was made between the actions of the same quantity of nitrogen added to the blood in the form of glycine, alanine, ammonium lactate and ammonium carbonate. The quantity of nitrogen employed was 56 mg. The choice of this dose arises from the fact that the first experiments were performed with the addition of 300 mg. glycine and must therefore be described as rather a casual one. The dose employed may be considered fairly moderate. The total volume of perfusion blood and liver tissue in the experiments amounted to 300—350 c. c. Provided the distribution was equal, the increase of the amino-nitrogen in the blood would thus be 16 to 19 mg. per cent. However, direct determinations of the amino-nitrogen in whole blood by Folin's method have shown that on an average the increase amounts to only about 8 mg. per cent., due to a large part of the amino acid added becoming fixed in the liver tissue. For this reason determinations of the blood amino-nitrogen provide a somewhat uncertain picture of the course of the amino acid breakdown, and even though determinations of this kind were made in the majority of the experiments, the results will not be discussed here.

The curves fig. 1 reproduce the typical course of the variations in the oxygen consumption after the addition of glycine, alanine, ammonium lactate and ammonium carbonate respectively.

The somewhat lower and more protracted course described by the increase of the oxygen consumption after the addition of glycine is a constant feature, one that may even be much more pronounced than is the case in the experiment reproduced in fig. 1. The longer the duration of the increase in the oxygen consumption, the greater is the uncertainty of the determination of the total extra consumption of oxygen. Experiments such as those described above have the advantage over experiments on intact animals that the increase in the oxygen consumption in per cent of the basal consumption, is large and at the same time of short

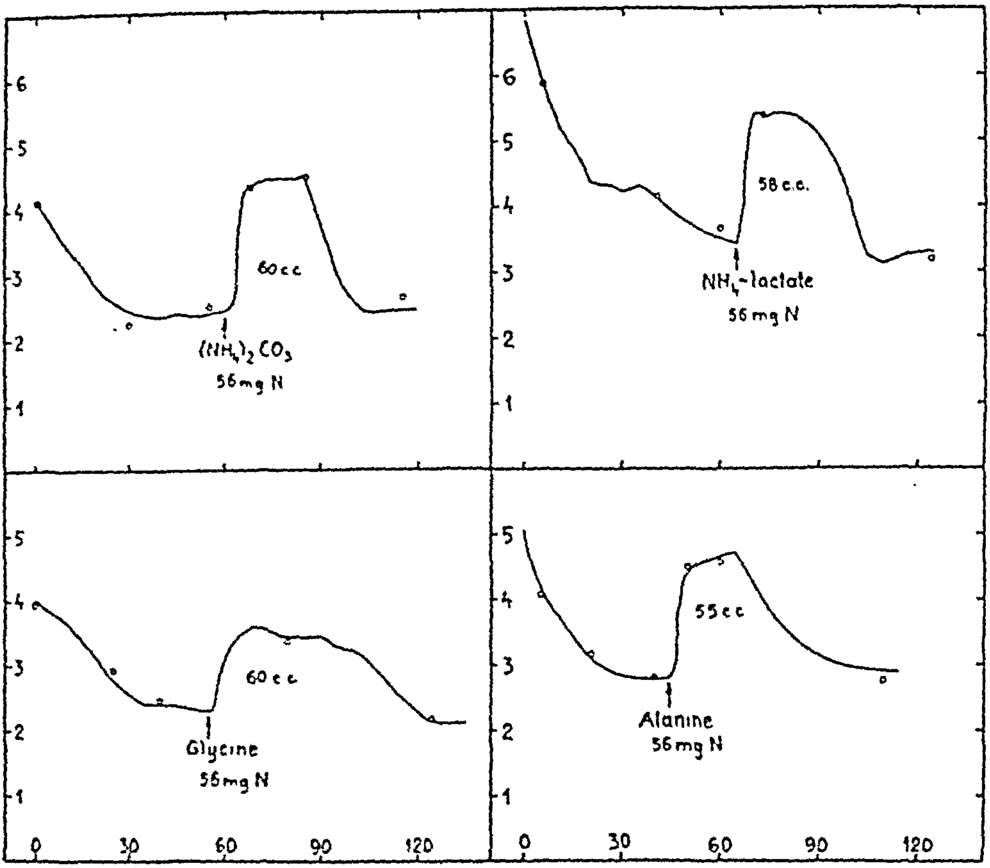


Fig. 1. Oxygen consumption in isolated liver preparations before and after addition of: 1. glycine, 2. alanine, 3. ammonium lactate, 4. ammonium carbonate. Abscissa: time in minutes. Ordinates: oxygen consumption per minute in c. c. O: VAN SLYKE determinations of oxygen consumption.

Table I.

Total extra consumption of oxygen (in c. c.) after addition of 56 mg. nitrogen to isolated liver preparations in the form of glycine, alanine, ammonium lactate and ammonium carbonate.

	Glycine	Alanine	NH_4-lactate	NH_4-carbonate
	60	55	46	60
	—	50	60	55
	40	58	55	47
	40	39	56	54
	53	43	58	
	70	63	38	
	57	65	53	
	60	55	53	
	47	52	52	
		45	48	
Average	53.5	52	52	54

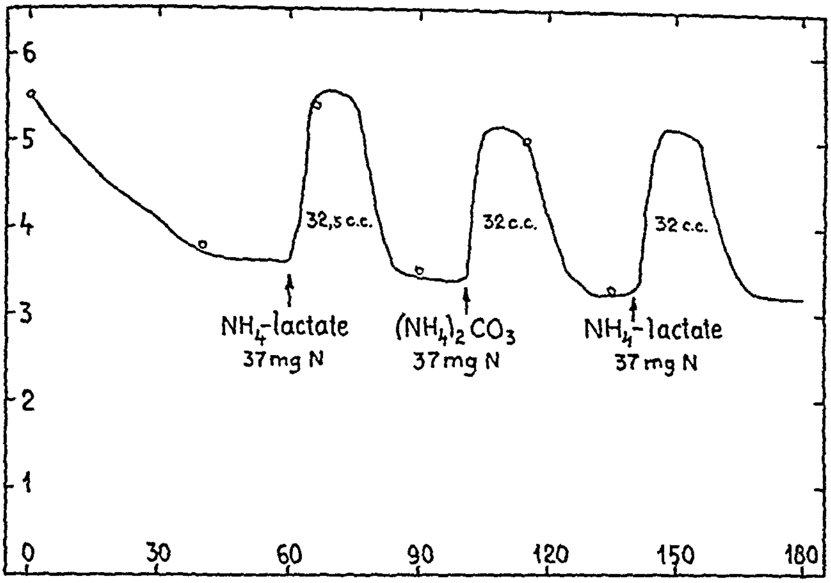
duration, which means that determination of the total extra consumption can be made with considerable accuracy.

However, the total extra consumption of oxygen varies more from experiment to experiment than would appear from the experiments shown in Fig. 1. Table I shows the total extra consumption of oxygen in all experiments performed with the addition of 56 mg. N.

When calculating the average total extra consumption of oxygen after the addition of glycine I omitted one experiment in which there was no definite increase of the consumption at all. This experiment is of particular interest, in that the determinations of the blood aminoacid-nitrogen showed that after having increased in the usual manner after the addition of glycine, the amino-nitrogen remained practically constant, whereas the normal is that it decreases and reaches almost the initial value when the increase of the oxygen consumption has subsided. Thus it would seem that the cause of the absence of any increase in the oxygen consumption was that for some unknown reason the liver did not metabolize the amino acid that was added. Accordingly the experiment supports the generally recognized opinion that a rise in metabolism is associated with metabolic changes undergone by the amino acids. In this connection I would mention that amino acids have no effects on the oxygen consumption in an artificially perfused hind-leg preparation.

As will be seen from Table I, there is fairly good conformity among the average values of the absolute extra consumption of oxygen after ingestion of the same quantity of nitrogen in the form of glycine, alanine, ammonium lactate and ammonium carbonate respectively. The same conformity appears in the experiment reproduced in fig. 2, in which three consecutive increases of the oxygen consumption in the same liver were produced by the addition of ammonium lactate, ammonium carbonate and then ammonium lactate again.

In this experiment a somewhat smaller quantity of nitrogen was employed for the purpose of obtaining increases in the oxygen consumption of short duration, as otherwise the period of the experiment would be too long. The fact that the duration of the increased oxygen consumption varies with the size of the dose much more than the maximum increase in the oxygen absorption per minute is very pronounced and appears distinctly in the experiment shown in fig. 3.



Fig, 2. Oxygen consumption of isolated liver preparation before and after consecutive additions of ammonium lactate, ammonium carbonate and ammonium lactate. Abscissa: time in minutes. Ordinate: oxygen consumption per minute in c. c. o: VAN SLYKE determinations of oxygen consumption.

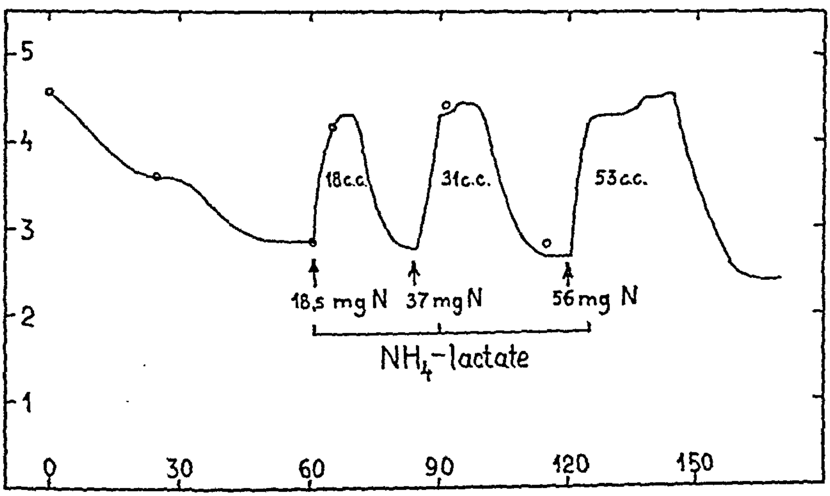


Fig. 3. Oxygen consumption of isolated liver preparation before and after addition of increasing amounts of ammonium lactate. Abscissa: time in minutes. Ordinate: oxygen consumption per minute in c. c. o: VAN SLYKE determinations of oxygen consumption.

It will be seen from the curve in fig. 3 that when ammonium lactate is added in the proportions of 1 : 2 : 3, one obtains with each addition an extra consumption of oxygen that is very nearly proportional to the quantity added, and that this is due mainly to the fact that the duration of the action increases with rising doses. This is in accordance with the result of previously published experiments on the feeding of increasing doses of protein to a dog (LUNDSGAARD 1931). There is reason for emphasizing this fact, as it provides a very simple explanation of the "summation experiments" of D. RAPPORT and collaborators (1924, 1926 and 1927), as already indicated in my earlier publication (1931).

The proportionality between the added quantity of nitrogen and the total extra consumption of oxygen also appears from Table II. As only a limited number of experiments were performed with the addition of smaller nitrogen quantities than 56 mg., all experiments with the same quantity of nitrogen are gathered into the same column, regardless of whether the nitrogen was supplied in the form of amino acid or ammonium salts.

Table II.

Total extra consumtion of oxygen (in c. c.) after addition of 18.5, 37 and 56 mg. nitrogen to isolated liver preparations.

	18.5 mg. N.	37 mg. N.	56 mg. N.
	17	37	
	12	33	
	18	23	
	18	31	
	16	26	cf.
		32	table I.
		32	
		33	
		31	
		28	
		35	
		39	
Average	16	32	53

The average extra consumption of oxygen per mg. of added nitrogen after the addition of 18.5, 37 and 56 mg. N. was 0.87, 0.87 and 0.92 c. c. Thus it may be said the experiments show that the total extra consumption of oxygen is proportional to the added quantity of nitrogen.

It was stated above that the increase of the oxygen consumption after the addition of glycine is as a rule more protracted in

its course than after the addition of alanine and particularly after that of ammonium salts. Without doubt this has some connection with a slower deamination and the consequent slower synthetization of urea after the addition of glycine. In my experiments only two samples were taken for urea determination after the addition of an amino acid or an ammonium salt, viz. 30 and 60 minutes after the addition was made. Consequently, the results of the experiments provide no exact measure of the rate at which urea is synthetized, particularly as the total blood volume varied somewhat in the different experiments. Nevertheless the increase in blood urea in mg. per cent. in the course of the first half hour after the addition must give an approximative measure of the rate at which the urea synthetization proceeded in the various experiments. If this increase of urea is plotted in a coordinate system against the maximum increase of the oxygen consumption expressed in c. c. oxygen per minute, it should be possible to form an idea of whether there is any relation between the rate at which urea synthetization takes place and the "pitch" of the increase in oxygen consumption. This was done in the diagram fig. 4.

As fig. 4 shows, there seems to be rather close proportionality between the rate of the urea synthetization and the maximum increase of the oxygen consumption after ingestion of the same quantity of nitrogen. As the total extra consumption of oxygen after ingestion of the same amount of nitrogen is rather constant, this means that the duration of the increase in the oxygen consumption is in inverse ratio to the velocity of the urea synthetization. This certainly seems to suggest a very close connection between increased oxygen consumption and urea synthetization.

In conclusion I may mention that experiments with the addition of ammonium chloride did not give results that were in complete accord with those obtained with ammonium lactate and ammonium carbonate. After the ammonium chloride has been added a steep increase of the oxygen consumption sets in very quickly as in the case of the other ammonium salts, but this increase soon turns into a fall in the oxygen consumption, which continues until values are reached far below the level prior to the addition. The explanation of this must be that the change in blood reaction which occurs when the ammonium ion is transformed into urea brings about a depression of the oxygen

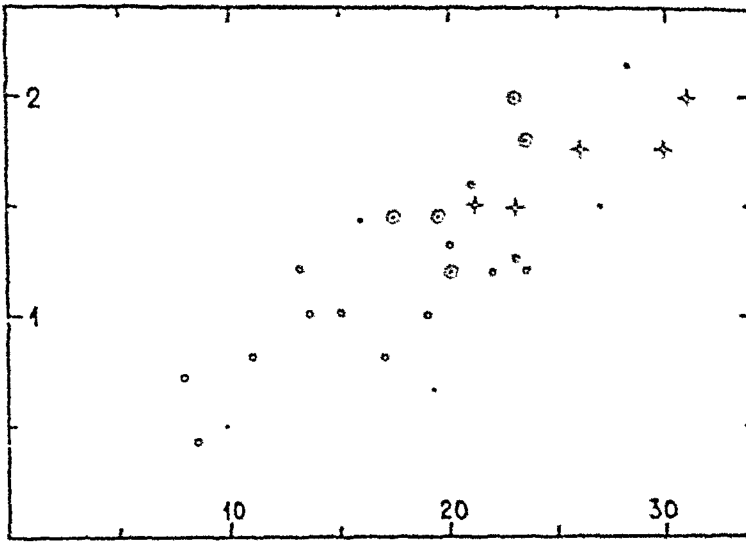


Fig. 4. Relation between maximal increase in oxygen consumption per minute (ordinate) and increase in blood urea (mg. per cent) in the first half hour period (abscissa). o: experiments with glycine, ⊙: experiments with alanine, ✢: experiments with ammonium lactate.

consumption which in part compensates the increase in the oxygen consumption. Ammonium lactate and ammonium carbonate are incapable of producing a corresponding increase of the hydrogen ion concentration, as the lactic acid is oxydized and converted into glycogen and the carbon dioxide is removed in the oxygenator.

I am unable to offer a satisfactory explanation to the discrepancy between OBERDISSE's and my results as to the effect of ammonium salts. According to my experience dog livers are less suitable for perfusion experiments than cat livers. OBERDISSE has performed only a few (4) experiments with ammonium carbonate. In two of the experiments the dose employed was rather small. In the experiment stated in detail in OBERDISSE's paper the ammonium carbonate was not added to the preparation till two hours after the beginning of the artificial perfusion. The oxygen consumption of the liver was very low and moreover markedly declining in the period just before the addition of ammonium carbonate was made.

Discussion.

The results of the above-described experiments with isolated liver preparations are in conformity with my earlier results (1931)

of experiments on intact animals. Both series confirm the original observation of GRAFE (1916) that ammonium salts have a specific dynamic action. In my experiments this action has proved to be of the same order as the action of amino acids. It would therefore seem that we must abandon all theories as to the cause of the specific dynamic action of proteins, according to which the action is associated with the nitrogen-free part of the amino acid molecule. An inorganic ammonium salt such as ammonium carbonate has a specific dynamic action, as has ammonium lactate, whereas lactic acid and sodium lactate have no effect on the energy output.

The possibility that the amino acids as such act as a metabolism-increasing irritant can only be maintained if at the same time we assume that ammonium salts have a similar action. Quite apart from the fact that with the exception of BORNSTEIN (1929) most authors who have occupied themselves with the specific dynamic action of proteins and amino acids have rejected this possibility, it is also possible in the experiments reported above to find arguments against such an idea. That the action of amino acids is associated with their transformation is suggested by the one experiment in which no effect was observed on the oxygen absorption after the addition of glycine, for, as I have stated, the determinations of the blood amino-nitrogen showed that the liver in question was unable to metabolize the ingested amino acid. The connection between the course of the increase of the oxygen absorption and the rate of the urea synthetization also argues very decidedly that the effect on the oxygen absorption is associated with a break down of the amino acids.

It should be possible to rule out the idea that the effect is associated with the deamination process itself, seeing that ammonium salts have the same effects as amino acids.

On the basis of the experiments described in the present article I consider that there is no room for the possibility that the ammonia freed by deamination should act as a metabolism-increasing irritant, though in my earlier publication on the same subject I was not prepared to deny that possibility. An effect on the nervous system — it was this possibility which formerly I believed should be taken into consideration — is out of the question when the effect can be produced on isolated liver preparations. Furthermore, it is certain that when the amino acids and the ammonium salts are added directly to the blood in a single dose,

as in these experiments, the ammonia concentration obtained *in blood and tissue* will be quite different according to whether *the addition* is an amino acid or an ammonium salt. As there is practically no difference in the increase in oxygen consumption after the addition of alanine and an ammonium salt, there cannot possibly be any parallelism between the ammonia concentration and the increase in the oxygen consumption. After the ingestion of an ammoniumsalt the ammonia concentration in the blood must reach a maximum at once, and thereafter decrease towards the very low, normal values. On the other hand, the course of the increase in the oxygen consumption is such that it very quickly reaches a maximum, whereupon it remains more or less unchanged for some time, to decrease rapidly to the same level as before the addition. Here again it is seen that increased oxygen consumption and ammonia concentration cannot have a parallel course. I have convinced myself that the total increase of the consumption of oxygen is not dependent on the concentration of the added substance, but solely on the absolute quantity added, by proceeding in the following manner in a few experiments. When ammonium lactate was added to the blood there was a rise in the oxygen consumption. After this increase had subsided the quantity of blood in the apparatus was reduced to about half. To this again the same quantity of ammonium lactate was added. Although the ammonium lactate concentration after the second addition was about twice as high as after the first addition, the rises in the oxygen consumption after the two additions were completely similar both as to course and as to the total extra consumption of oxygen.

In my opinion the experiments described above can have only one interpretation, viz. that the specific dynamic action of amino acids is associated with urea synthetization, which apparently requires a considerable amount of energy. We know from KREBS' experiments (1933) that urea synthetization proceeds only under aerobic conditions and therefore must be assumed to be brought about by oxydative processes.

As the total extra consumption of oxygen has been determined with fairly high accuracy in these experiments, thanks to the continuous registration of the oxygen consumption and the relatively short duration of the increases, it should be possible, on the basis of the experiments, to estimate the energy consumption associated with the urea synthetization.

The average extra consumption of oxygen per gramme of added nitrogen in my experiments is 900 c. c., which corresponds to about 4.5 calories. This figure, however, cannot directly be accepted as a measure of the amount of energy consumed per gramme of nitrogen converted into urea. It is not certain a priori that the whole quantity of nitrogen added is transformed, and transformed into urea within the period in which the absorption of oxygen is increased. Even if this were so, it is not certain that the added quantity of nitrogen gives a measure of the increase in urea synthetization within that period. Without the addition of amino acids or ammonia salts there is a synthetization of urea in the isolated liver, and this spontaneous synthetization might possibly be impeded after the addition of a surplus of substrate.

As already stated, a considerable part of the ingested quantity of amino acid is fixed in the liver tissue, and therefore determinations of the amino-N content in the blood are not very suitable for an evaluation of whether the whole of the added quantity of amino acid is transformed within the period during which the oxygen absorption is increased. Determinations of the blood amino-N before and after the addition of glycine have shown that one hour after the addition the amino-N content is usually from 1 to 1.5 mg per cent higher than before the addition. However, a slight increase in the amino-N content in the blood may also occur in liver perfusion experiments in which no amino acids are added to the blood.

There is no doubt that we obtain better information about the metabolism of the amino acids by comparing the increases in the blood urea concentration occurring after the addition of the same amount of nitrogen as amino acid and as ammonium salt. An hour after the addition of an ammonium salt in the quantities employed the added quantity of ammonia has disappeared completely from the blood, so that it must be justifiable to assume that the entire quantity is converted into urea. The formation of other nitrogenous waste products is not very probable.

In my experiments the determinations of blood urea were always made immediately prior to the addition of amino acid or ammonium salt and further 30 minutes after and 60 minutes after. In experiments with ammonia salts, too, the last urea determination was made an hour after the addition, despite the fact that the increase in the consumption of oxygen had usually subsided after only 40—45 minutes. In eight experiments with

glycine (56 mg. N.) the blood urea concentration in the course of an hour after addition rose on an average 33.6 mg per cent. The corresponding values for nine experiments with alanine and seven with ammonium lactate (56 mg. N.) were 30.0 and 33.7 mg per cent respectively. As the quantity of blood and the liver volume could not be kept quite constant from one experiment to the next, I am inclined to consider this conformity as being so good that one should be able to conclude from these urea determinations that nitrogen, added in the form of glycine and alanine, is transformed just as completely into urea as nitrogen in the form of ammonia salt; presumably this again means that the amino-nitrogen is converted completely into urea.

The increase in blood urea after the addition of ammonium lactate corresponds to 15.7 mg per cent of urea-nitrogen. As the addition amounted to 56 mg. N., it should be possible to obtain a rise of 16 mg per cent if the volume of the "solvent" were 350 c. c. As was stated above, the quantity of blood plus the liver volume in the experiments amounted to 300—350 g. There would thus seem to be conformity between the quantity of nitrogen added and the observed rise of the blood urea-nitrogen. The condition of this conformity is, however, that after the addition of ammonia lactate (or one of the amino acids) urea is formed only from the added substrate — in other words that the spontaneous urea formation ceases. The fact is that this spontaneous formation is not at all inconsiderable. In numerous experiments performed at this institute with artificially perfused cat livers the rise of the blood urea concentration per hour has averaged 13 mg per cent, or 6.1 mg per cent of urea-nitrogen. If a spontaneous urea formation of this order had also taken place in the period of about an hour in which the added amino acids and ammonium salts were metabolized in the liver, the increase in urea-nitrogen would have been 22.1 mg per cent as compared with the observed increase of 15.7 mg per cent. It must therefore be assumed that the "spontaneous" urea formation ceases in the presence of ample substrate. This idea will not seem unreasonable, as there must be a limit beyond which the intensity of urea synthetization cannot be raised by increasing the supply of substrate. If my main conclusion is accepted, i.e. that the rise of the oxygen consumption after the addition of amino acids and ammonia salts is associated with the urea synthetization, direct support for the aforesaid idea is to be seen in the fact that the

addition of increasing quantities of ammonium salt will give no noteworthy increase in the rise of oxygen consumption per minute, but merely an increase in the duration of that rise.

If the extra consumption of oxygen must be put in relation not to the total urea formation in the period in which the oxygen consumption is increased, but to the difference between this formation and the spontaneous formation in a period of the same length, the energy consumption per gramme of nitrogen transformed into urea will be much greater than the ca. 4.5 calories calculated above, viz. 7.4 calories. This latter figure lies very close to the one originally given by RUBNER, whose calculation was 31.5 per cent. of the physiological combustion heat of the proteins, or 8 calories per gramme nitrogen.

The question concerning which of the two modes of calculation is the right one is of almost decisive importance. For if the latter mode is the right one it means that the specific dynamic action of the amino acids, and with them the proteins, consists solely of an action on the oxygen consumption of the liver, which to my mind would again mean with an energy consumption in conjunction with the urea synthetization. It is true that in RUBNER's calculation of the specific dynamic action of the proteins (8 calories per gramme nitrogen) no allowance is made for the protein metabolized by the experimental animals during the days of inanition which, if the calculation is to correspond to the one employed above, should be deducted from the protein metabolized in the protein-fed days. The consequent correction of RUBNER's figure can, however, only lead to an increase of about 10 per cent. on the specific dynamic action of the proteins. Even taking this correction into account, my figure of 7.4 calories per gramme of nitrogen comes so near to RUBNER's that my result must be said to indicate that the cause of the specific dynamic action of the proteins must, at any rate mainly, lie in an energy consumption in association with the synthetization of urea. In that case other factors, such as the action of amino acids on the oxygen consumption of the kidneys as demonstrated by OBERDISSE (1940), must at most play a very subordinate rôle.

It is difficult to say with certainty which of the two methods of calculation of the relation between urea synthetization and the extra consumption of energy is the more correct. For myself I consider it to be the latter method, by which the extra consump-

tion of energy is placed in relation to the increase in urea synthetization in excess of the spontaneous formation.

As I have said, and as will appear clearly from the curves, the oxygen consumption of the liver in the first period after the commencement of artificial perfusion falls rapidly. Generally the oxygen consumption in the course of 45 to 60 minutes adjusts itself at a level of about 60—50 per cent. of the consumption recorded when measurement begins 10 to 15 minutes after perfusion commences. To me there is no doubt that the high oxygen consumption observed immediately after the commencement of artificial perfusion expresses the normal oxygen consumption of the liver. The question might therefore be asked as to whether the same increases in oxygen consumption after the addition of amino acid would be observed if the liver's oxygen consumption remained normal. The question evades direct answer, as it is impossible to determine even approximately the extra consumption of oxygen caused by the amino acid in the period when the consumption is falling rapidly. I can merely say that in the various experiments the induced extra consumption was quite independent of whether the oxygen consumption to which the liver had adjusted itself were a little higher or lower.

I have as yet been unable to find the reason for the pronounced fall in the oxygen consumption of the liver in the first period after the latter's isolation, despite numerous attempts to throw some light on the problem experimentally. Nor has it therefore been possible to keep the liver's oxygen consumption up to the presumed normal level observable immediately after isolation. At the present moment one can merely say that the initial high consumption cannot be due to an oxygen debt accumulated during preparations for artificial perfusion. Normally the interruption of the circulation through the liver does not exceed half a minute, and deliberate interruptions of the circulation for several minutes after commencing artificial perfusion lead merely to quite transitory increases in the oxygen absorption when circulation is resumed. Nor is the drop in the oxygen consumption explained by the fact that perfusion proceeds only through the portal vein, as there is a corresponding fall in the consumption in preparations perfused simultaneously through the portal vein and the hepatic artery. I hope that in a later publication I shall be able to return to the interesting question of whether the reduction of the oxygen

consumption is due to the elimination of nervous influence, of substrate for oxydations, or of hormonal action.

Conclusion.

The cause of the specific dynamic action of amino acids, and therefore of proteins, lies in a consumption of energy in association with urea synthetization.

Summary.

In experiments with isolated cat livers amino acids (glycine and alanine) and ammonium salts (lactate and carbonate) bring about an extra consumption of oxygen which is proportional to the added quantity of nitrogen and independent of whether the nitrogen is supplied in the form of amino acid or ammonium salt.

With increasing doses there is an increase especially of the duration of the rise in the oxygen consumption, whereas the maximum oxygen consumption per minute is increased only very slightly.

The maximum increase of the oxygen consumption per minute seems to depend on the rate of urea synthetization.

The total extra consumption of oxygen seems to be independent of the concentration of the added substances and to be governed solely by the absolute quantity added.

The total extra consumption of oxygen per gramme of added nitrogen averages 900 c. c. (about 4.4 calories).

If the extra consumption of oxygen is calculated not in relation to the added quantity of nitrogen, but in relation to the increased nitrogen metabolism within the period in which the oxygen consumption is increased, the oxygen consumption per gramme of extra-converted nitrogen is 1,640 c. c. (about 7.4 calories).

The oxygen consumption of the artificially perfused liver falls in the course of about an hour to 60—50 per cent. of the consumption immediately after isolation, and thereafter remains practically constant.

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The State of Bile Salt Solutions.

I. Introduction.
II. Conductivity measurements on dilute solutions of sodium taurocholate at 25° C.

By

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I. Introduction.

The bile salts play an important physiological rôle in digestion. Resorption of fats (in the broadest meaning of the word, including fat soluble vitamins *etc.*) in the small intestine can take place only if bile salts are present. In the case of neutral fat (triglycerides), which constitute the bulk of the fatty material, the presence of a lipase is also necessary. The lipase is activated by the presence of bile salts and splits the neutral fats into glycerine and fatty acids. At the hydrogen ion concentration in the small intestine the fatty acids cannot exist as sodium salts[1] and the free acids are insoluble. The free acids are emulsified and to some extent dissolved by the sodium salts of the conjugated bile acids and by these means transported to the mucosa. The power of solutions of bile salts to dissolve otherwise insoluble fatty material into clear solutions which contain the dissolved material in a diffusible form, *i. e.* the "hydrotropic" action of the bile salts, is evidently of great physiological importance but the actual mechanism does not yet appear to have received a satisfactory explanation.

VERZAR and his collaborators (*cf* VERZAR and McDOUGALL 1936, pp 161—168) have studied the power of the most common conjugated bile salts, *i. e.* sodium glycocholate and sodium taurocholate, to dissolve free fatty acids under varying conditions. They found that if the amount of fatty acid was small optically clear solutions were obtained which contained the fatty acid in diffusible

[1] Statements as to the existence of sodium soaps in the small intestine may still be found in recent textbooks of physiology, *cf. e. g.* REIN (1940).

an interior consisting of the hydrocarbon chains in a liquid state and a surface made up by the hydrophilic end groups and their attached "gegenions". The micelle has a diameter somewhat larger than the double length of the hydrocarbon chain of the paraffin chain salt (HARTLEY and RUNNICLES 1938).

The appearance of micelles takes place rather suddenly and is accompanied by comparatively sharp changes in many physical properties of the solutions. The critical concentration may thus be found from measurements of the variation of equivalent conductivity (EKWALL 1932, LOTTERMOSER and PÜSCHEL 1933, McBAIN, DYE and JOHNSTON 1939), surface tension (LOTTERMOSER and STOLL 1933, POWNEY and ADDISON 1938), hydrolysis (POWNEY and JORDAN 1938), osmotic pressure (HESS and SURANYI 1939), density (TARTAR and WRIGHT 1939, WRIGHT, ABBOTT, SIVERTZ and TARTAR 1939), viscosity etc. with concentration. The micelles are below ultramicroscopical size and they may appear in optically clear solutions.

In more concentrated solutions, above 0.1—0.2 N, another type of aggregation may appear. These colloidal aggregates have probably a liquid crystalline structure, are comparatively large and not spherical. Solutions containing such aggregates give x-ray diffraction patterns (THIESSEN and SPYCHALSKI 1931, HESS, PHILIPOFF and KIESSIG 1939, STAUFF 1939). STAUFF calls the micelle first formed (HARTLEY's micelle) "Kleinmizelle" and the larger crystalline micelle "Grossmizelle".[1] LAWRENCE (1935) speaks of secondary micelles. In solutions containing micelles there has appeared a new phase *i. e.* the hydrocarbon phase in the interior of the micelles. This is responsible for the power of soap solutions not only to emulsify hydrophobic compounds such as hydrocarbons but also to a certain extent to dissolve them, the water solutions remaining clear (PICKERING 1917, SMITH 1932, HARTLEY 1937, LAWRENCE 1937). The hydrocarbon dissolves in the hydrocarbon interior of the micelle.

Despite the fact that several polar groups are distributed over the hydrocarbon skeleton of the bile acid molecule (the most common bile acid, cholic acid, is shown in fig. 1) the bile salts are unsymmetrical electrolytes as is shown by the low surface tension of their solutions. The ionized carboxyl is the most water attracting group in the molecule and outweighs the three hydroxyl groups, thus causing the electrolytical asymmetry.

[1] In the following we use the word "micelle" in the meaning "Kleinmizelle".

The hydroxyl groups are important, however, as they increase the water solubility of the bile salts. Sodium cholate may be obtained in clear water solution in a concentration of 1 N at room temperature. Such concentrated solutions are very viscous.

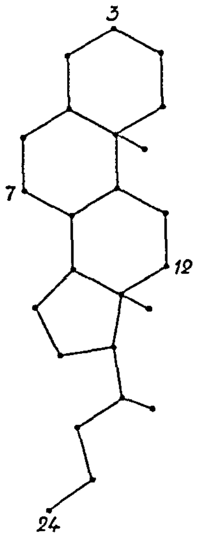

Fig. 1. Carbon skeleton of cholic acid ($C_{24}H_{40}O_5$). Each point represents a carbon atom. Carbon atoms 3, 7 and 12 carry hydroxyl groups, 24 is the carbon atom of the carboxyl group. In taurocholic acid the carboxyl · — COOH at 24 is replaced · by — CONHCH$_2$CH$_2$SO$_2$OH. The somewhat unusual projection is chosen to show the elongated form of the molecule.

As the bile salts are unsymmetrical electrolytes we might expect some form of aggregation, similar to that of the paraffin chain salts, to occur at higher concentrations. This has already been suggested by HARTLEY (1936). Some differences may, however, be expected by the presence of hydroxyl groups and by the fact that the condensed ring system of the bile acids (the *cyclo*pentanoperhydrophenanthrene skeleton) must be considerably less flexible than the hydrocarbon chain of the paraffin chain salts. With respect to aggregation and micelle formation the bile salts might be expected to occupy an intermediate position between the paraffin chain salts and certain dyes which show colloidal properties. The latter have been studied especially by ROBINSON and his collaborators (1939).

In order to determine the rôle of micelle formation for the hydrotropic action of bile salts a knowledge of the constitution of their water solutions is necessary.

Rather little is at present known in this respect. BASHOUR and BAUMAN (1937) mention, without giving any experimental details, that the equivalent conductivity curve of sodium cholate does not show any discontinuity indicating aggregation.

ROEPKE and MASON (1940) have recently measured the equivalent conductivity and osmotic coefficients of solutions of sodium cholate, sodium glycholate and sodium desoxycholate with the view to study micelle formation. Their work may be criticized on several grounds, however. In the first place they appear to have used a commercial sample of cholic acid without purification. It is well known that such preparations may contain

considerable amounts of other bile salts and fatty acids (SOBOTKA 1938). They state nothing about the desoxycholic acid used and give no analyses. Secondly, their method of making up solutions for conductivity work does not appear very satisfactory and measurements have not been carried out with sufficient precision in the dilute range (0.005—0.05 N), where micelle formation may be expected to begin. Also, cholic acid ($K_s = 6.46 \times 10^{-6}$), glycocholic acid ($K_s = 35.5 \times 10^{-6}$) and glycodesoxycholic acid ($K_s = 104.7 \times 10^{-6}$) are all weak acids and solutions of their sodium salts are subject to hydrolysis which may completely svamp the effect of micelle formation on the equivalent conductivity-concentration curve. In order to study micelle formation in solutions of bile salts, it would appear more suitable to use sodium taurocholate or other tauroderivatives which are salts of strong acids (taurocholic acid $K_s = 2.75 \times 10^{-2}$) the alkali salt solutions of which are not subject to hydrolysis. (The values for the dissociation constants are from JOSEPHSON (1933), see, however, also KUMLER and HALVERSTADT (1941). No values for the critical concentrations for micelle formation can be obtained from the work of ROEPKE and MASON and with regard to the possible rôle of micelle formation for the hydrotropic action of the bile salts they state: "While it cannot be definitely stated at this time that the solution of water-insoluble substances by the bile is associated into aggregation of bile salt ions, such a conclusion appears reasonable."

Some more insight is gained into this question by the recent work of MCBAIN and collaborators (1941) who studied the solubility of a fat soluble dye and of chlorophyll in solutions of commericial sodium desoxycholate. They found that below about 0.005 N the solubility of the dye (moles dye/moles NaDe) was very small. Between 0.005 and 0.09 N the solubility increased twenty-fold and above 0.09 N it remained fairly constant. The results are interpreted on the assumption that micelle formation starts at 0.005 N although micelle formation in dilute solutions of pure desoxycholate solutions has not yet been demonstrated.

It was observed, however, already in very early measurements of the surface tension of bile salts such as sodium taurocholate (*cf* SOBOTKA 1938) that the surface tension — concentration curve exhibited a minimum at about 0.01 N. At the time this could not be given an adequate explanation, but more recent measure-

ments on paraffin chain salts (alkyl sulphates) (LOTTERMOSER and STOLL 1933) have given the same type of curve and it is now known that the minimum occurs at the critical concentration for micelle formation. From the old surface tension measurements *a crititical concentration of about 0.01 N might thus be* expected for sodium taurocholate and this is also the value found by the conductivity measurements to be described.

The optical rotation of bile salts increases with decreasing concentration and JOSEPHSON found that this increase was much more rapid below a certain concentration, which in the case of sodium taurocholate was about 0.75 per cent, *i. e.* about 0.015 N. JOSEPHSON (1935) considered that this indicated "some change in the molecule". It appears probable that the change is due to micelle formation.

II. Conductivity measurements on dilute solutions of sodium taurocholate at 25° C.

Measuring equipment.

The bridge. A completely shielded bridge similar to that described by SHEDLOVSKY (1930) was used (Fig. 2). For the theory and use of alternating current bridges of this type the papers of JONES and JOSEPHS (1928) and SHEDLOVSKY (1930) should be consulted. The fixed ratio arms R_1 and R_2 consisted of two closely matched resistors of 2,000 ohms each of the Leeds & Northrup wowen type. The measuring resistance M consisted of Leeds & Northrup shielded decade resistance box No. 4750 having six decades from 10 × 0.01 to 10 × 1,000 ohms. Resistances greater than 11,111 ohms were measured by adding extra resistors (10,000 and 20,000 ohms) of the L & N wowen type in series with the decade box, or, in some cases, by shunting the cell with a 10,000 ohm resistor. The decade resistance box was compared with a set of precision resistors in the Institute of Chemistry, Uppsala, by Dr. I. HEDLUND, to whom our thanks are due. In the five lower decades, no error larger than 0.03 ohm was found. The largest error found in the 10 × 1,000 ohm decade was 2.1 ohm at the

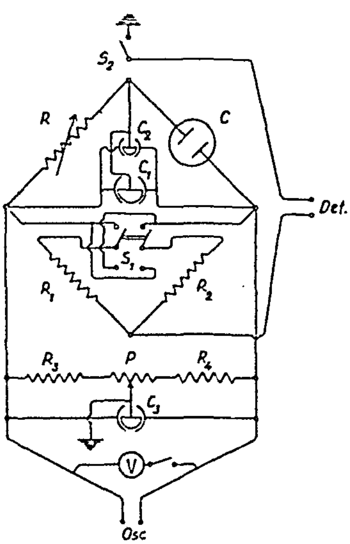

Fig. 2. Circuit diagram of the bridge.

10,000 ohm setting (0.02 per cent). The zero resistance of the decade box (all dials at zero) was found to vary from 0.035 to 0.08 ohm, it was usually 0.05 ohm. The largest error found in the separate 10,000 and 20,000 ohms resistors used for measuring resistances above 11,000 ohms were of the order of 0.02 per cent. The appropriate corrections were applied in the measurements. The resistors R_3 and R_4 of the Wagner earth connection were similar to R_1 and R_2 and of 2,000 ohms each. The potentiometer P (General Radio) had a resistance of 20 ohms. The differential condensors C_1 (250 cm. capacity each side), C_2 (15 cm.) and C_3 (250 cm.) were built from standard radio parts. General Radio low capacity switches were used.

The bridge was fed from a battery driven push-pull oscillator through a shielded transformer (Svenska Radioaktiebolaget). Frequencies from 700 to 3,000 cycles per second could be chosen at will and the amount of harmonics was negligible. The input to the bridge was usually 2 to 3 volts and could be directly measured by means of the high resistance rectifier voltmeter V (Siemens) shown in Fig. 2. The detector consisted of a two-stage transformer coupled, battery driven amplifier preceded by a transformer and followed by head phones.

A relative precision of 0.01 % or better is readily obtained with the bridge when measuring electrolytic resistances of 1,000 ohms or more. The accuracy of course depends on the care with which the measuring resistors have been calibrated.

Thermostat. A large oil bath containing transformer oil was used as thermostat. The cells were fixed in the bath by a clamp around the flask neck and immersed in the oil to the dotted line in fig. 3. An electric heater, contact thermometer and relay together with efficient stirring kept the temperature constant to $\pm$ 0.01° C. The temperatures were checked by a calibrated thermometer. A temperature variation of $\pm$ 0.01° gives rise to an error of $\pm$ 0.02 % in the conductivity measurements. This error is unnecessarily large and a new thermostat giving better temperature control is under construction.

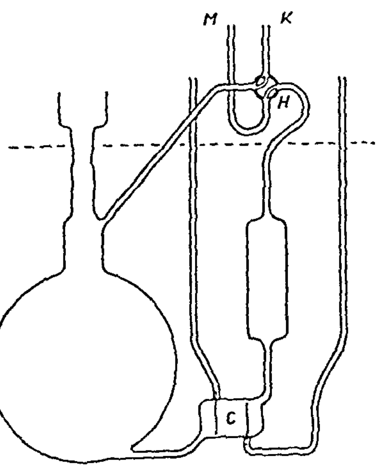

Fig. 3. Flask cell.

Conductivity cells. Cells of the JONES and BOLLINGER (1931) type were coupled to flasks of 500 or 1,000 cc. capacity forming flask cells as shown in fig. 3. The cells were made from Jena "Geräte 20" or, in some cases, Jena "Normal 16III" glass. By applying a slight gas pressure at K the cell C may be filled or emptied by turning the stopcock H through 90 degrees. In- and outlets M and K may also be connected together and the mixing of the contents in the cell C and the flask carried out by turning the whole

cell around an axis normal to the plane of the paper in fig. 3. The cells were platinized as described by JONES and BOLLINGER (1935). Cells having different cell constants were available. They were standardized by 0.1 and 0.01 demal potassium chloride prepared according to the directions of JONES and BRADSHAW (1933). The cell constants were checked at intervals with fresh potassium chloride standards.

Weight burettes of the type described by SHEDLOVSKY and BROWN (1934) made of Jena Geräte glass or transparent quartz and of 75 and 250 cc. capacity were used. The smaller burets were weighed on an analytical balance sensitive to 0.1 mg and the larger burets and the cells on a balance having a capacity of 3 kgs. and a sensitivity of 1 mg. The weights were compared with a set of weights calibrated by Kungl. Myntverket, Stockholm. Weights were corrected to vacuum.

Conductivity water was obtained by redistilling ordinary distilled water in a still of the Bourdillon-Weiland type as described by ELLIS and KIEHL (1935). The conductivity of the water was measured in a "Geräte" glass cell having unplatinized electrodes and a cell constant of 0.0734.

Water having a conductivity of $0.2—0.3 \cdot 10^{-6}$ ohm^{-1} cm^{-1} was easily obtained.

Material used.

Pure sodium taurocholate cannot be prepared from ox bile. The best natural sources are dog or fish bile. The measurements have been carried out with three different preparations of sodium taurocholate. Specimen I was from the collection of the late Professor OLOF HAMMARSTEN and had been prepared by him, probably from fish bile, during his classical investigations into the bile acids, carried out in this laboratory more than thirty-eight years ago (1904). The preparation, which had been kept in a tightly stoppered bottle, had a pure white, microcrystalline appearance and showed no sign of deterioration.

Specimens II and III were synthetic, prepared by coupling taurine with cholic acid according to the method of BONDI and MÜLLER (1906), as modified by CORTESE (1937). The cholic acid used was prepared from ox bile by the methods of HAMMARSTEN (1925) and LANGHELD (1908). The ethyl cholate was purified by repeated crystallization from ethyl acetate and melted at 161—163° C. The taurine used had been prepared from bile in this laboratory by the method of HAMMARSTEN (1925). The chlorine free specimens of sodium taurocholate were recrystallized twice from alcohol-ether and before used dried to constant weight over phosphorus pentoxide at 135° C.

Analysis.[1]

	C	H	N	S	Na	Rot. α_D^{20}
Calcd for $C_{26}H_{44}O_7NSNa$ (537.5)	58.1	8.25	2.60	5.95	4.28	—
Found specimen I . .	55.9	8.18	2.52	5.83	4.29	+ 23°.8
	56.3	8.25	2.56	5.83		0.463 g/10 cc
specimen II . .	54.2	8.61	2.70	5.68	4.35	+ 23°.6
	54.4	8.53	2.66	5.72		0.293 g/10 cc
specimen III . .	55.1	8.60	2.77	5.85	4.36	—
	55.4	8.64	2.72	5.80		

(The low C values are probably due to combustion difficulties, the filling of the combustion tube not being specially adapted to this difficultly combustible compound.)

Procedure.

The general procedure of SHEDLOVSKY (1932) was followed. A flask cell of Jena "Normal" glass having a cell constant of 18.00 was employed. The cell was carefully rinsed with conductivity water, dried by means of alcohol and ether and placed in the thermostat. The sodium taurocholate, previously dried to constant weight at 135° and kept in a vacuum desiccator over phosphorus pentoxide, was weighed up and placed in the flask, the flask filled with nitrogen and a suitable amount of (carbon dioxide-free) conductivity water added by means of the weight burette. After temperature equilibrium had been attained the conductivity cell was repeatedly filled and emptied and the resistance measured for each filling until a constant value was attained. A further amount of water was then added from the weight burette and the procedure repeated. In addition to the weight dilution series of measurements thus obtained individual points were obtained as a check on separate solutions made up by weighing. The mixing of the solution in flask cell has to be carried out carefully in order to avoid foaming. This is especially important for the most dilute solutions. If such a solution is shaken strongly a large part of the taurocholate is removed from the solution into the foam.

Results.

Fig. 4 shows the results. In the most dilute range the sodium taurocholate behaves as a normal electrolyte, the equivalent conductivity being a linear function of the square root of the concentration. The slope of this straight part is smaller than the theoretical slope required by the Onsager equation, however. Just above 0.01 N there is a sudden change in slope and the curve is nearly horizontal between 0.01 and 0.03 N after

[1] All analyses, except sodium, are microanalyses by Dr. MAX MÖLLER, Copenhagen. For the sodium analyses we are indebted to Dr. I. HEDLUND and Mr. W. KIRSTEN. Mr. KIRSTEN also measured the optical rotation.

which it again starts falling. The equivalent conductivity is around 60 in the region below 0.01 N and is only about half that of the alkyl sulphonates below the critical concentration (TARTAR and WRIGHT 1939, WRIGHT, ABBOTT, SIVERTZ and TARTAR 1939). As is seen from fig. 4, the curve for the Hammarsten preparation (specimen I) lies somewhat above that of our synthetic specimen II, but the two curves are of exactly the same form. The synthetic specimen III agreed rather closely with specimen

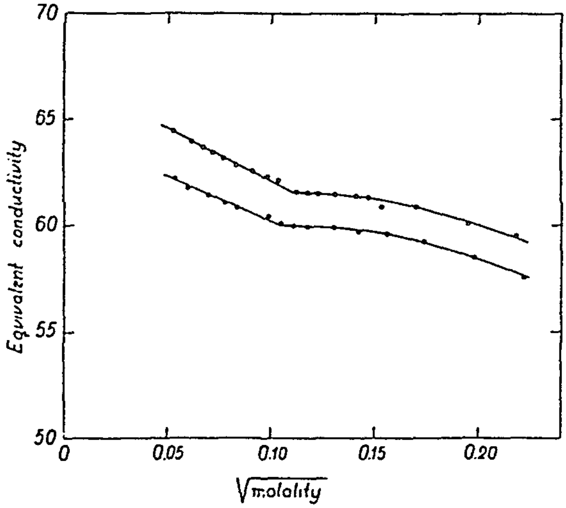

Fig. 4. Equivalent conductivity of sodium taurocholate as a function of the square root of the concentration.
Upper curve: specimen I (natural). — Lower curve: specimen II (synthetic).

II. The precision of the measurements and the reproducibility for each specimen is very good and is of the order of a few tenths of one per cent for freshly prepared solutions. We can at present offer no explanation for the difference between the natural and synthetic specimens. We have found that the equivalent conductivity is very sensitive to small amounts of impurity. The presence of sodium chloride or free taurine from the synthesis raises while the presence of fatty acids lowers the conductivity. The material is also rather hygroscopic and the different preparations, although dried to constant weight, may differ in water content. A table of the equivalent conductivity at different concentrations will therefore not be given until this point is clear. For the present purpose the form of the conductivity curve is more important than the absolute value of the equivalent conductivity. — It was found that the conductivity of dilute sodium taurocholate solutions that had been kept in the flask cell

for 24 hours or more increased slowly with time. Jena "Normal 16^{III}" glass belongs to the third hydrolytic class and it was therefore thought that the slow increase might be due to the glass. A check was therefore made with a Jena "Geräte 20" glass (first hydrolytic class) cell. The conductivity was now found to remain practically constant for 24 hours or more.

Discussion.

The sudden change in slope of the conductivity curve at about 0.01 N indicates that some change takes place in the sodium taurocholate solution at this concentration. The change is most probably due to the onset of aggregation, *i. e.* micelle formation. In solutions of alkyl sulphates (LOTTERMOSER and PÜSCHEL 1933) and alkyl sulphonates (TARTAR and WRIGHT 1939, WRIGHT, ABBOTT, SIVERTZ and TARTAR 1939) a sudden and very marked fall in the conductivity curve takes place at the critical concentration, while a simple aggregation might be expected to increase the conductivity, the frictional resistance of micelles being smaller than that of single molecules for the same amount of material transported (Stoke's law effect of McBAIN). HARTLEY and collaborators (1936, 1938) have shown by means of transport number measurements that the fall observed for the paraffin chain salts is caused by the large number of ions of opposite sign (gegenions) that are attached to the surface of the micelle. In the case of sodium taurocholate, although there is no absolute rise in the conductivity, there is a rise above that expected for simple ions (the curve in the region 0.01—0.03 N lies above the continuation of the straight part of the most dilute range). This may be due to the STOKE's law effect, the gegenions in this case being fewer than in the case of paraffin chain salts. Transport number measurements are necessary to determine whether or not this is the case.

The surface tension-concentration curve obtained by ETTISCH and KOGANEI (1928) shows a minimum at about 0.005 N while KRAJEWSKY and WWEDENSKY (1927) state that a minimum is obtained at 0.017 N. No analyses of the sodium taurocholate or any statements regarding its purity are given, however, and it is probable that in both cases impure preparations have been employed. Although there is thus an approximate agreement between the critical concentration derived from surface tension

and conductivity data it would appear that the surface tension data for sodium taurocholate are in need of revision.

The present demonstration of aggregation in dilute solutions of sodium taurocholate together with the solubility measurements of McBAIN, *et al.* (1941) dealt with in the Introduction is strong evidence for the "micelle solubility" theory of the hydrotropic action of bile salts. This theory, which considers that the presence of colloidal bile salts micelles is necessary for the transport function of the bile salts, is very different from the views of VERZAR (1936), who considers that molecular complexes are formed and that these complexes form a true molecular solution.

We gratefully acknowledge grants from the Andersson Foundation which made this investigation possible.

Summary.

The state of bile salt solutions in relation to their physiological transport function is being studied. The conductivity of sodium taurocholate is normal below 0.01 N (at 25° C), but at this concentration the conductivity curve suddenly changes its slope. The change is interpreted as being due to micelle formation. This view is supported by earlier measurements of the surface tension of sodium taurocholate solutions if these are interpreted in the light of more recent work on paraffin chain salts.

The demonstration of micelle formation in dilute solutions of bile salts together with recent solubility measurements of McBAIN and his collaborators is strong evidence for a "micelle solubility" mechanism for the hydrotropic action of bile salts.

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From the Biochemical Department of the Medical Nobel Institute, Stockholm.

Cystine and Methionine Determinations in Cytochrome c.

By

Å. ÅKESON.

(Received 8 August 1942.)

In previous reports from this institute (THEORELL 1936, THEORELL and ÅKESON 1941), it has been shown that cytochrome c contains 6 atoms of sulphur per molecule. Two of these belong to the cysteine-remains in porphyrin c (THEORELL 1939, 1940). Concerning the four other S-atoms contained in the protein-component nothing has hitherto been known. By way of supplementing previously published analyses of amino acids (THEORELL and ÅKESON 1941) a determination of the sulphur-containing amino acids is of a certain interest.

The determinations were carried out according to KASSEL's and BRAND's (1938) modification of BAERNSTEIN's method. According to this method, after hydrolysis of the substance at 150° C. with strong hydriodic acid, cystine and cysteine are determined iodimetrically as cysteine, while the methionine is determined in two ways, partly as methyl iodide and partly as homocysteine-thio-lactone, the two latter also iodimetrically.

Some control-determinations were carried out with pure amino-acids, in two of these with protohemin present. The results are given in table 1.

The methionine determinations give too low values according to both methods, and KASSEL and BRAND therefore introduced correction factors for these: for the methyl iodide titration f = 1,067, and for the homocysteine titration f = 1,120. Also for the cysteine titration they introduced a factor f = 1,023. According to the values in table I, the corresponding factors for the methyl iodide titration and for the homocysteine titration respectively will

Table 1.

Weighed		Methionine found		Cystine found	S found as H_2S & SO_2	Total-S found	
Substance	Amount	acc. to methyl iodide	acc. to homo-cyst-eine			acc. to methyl iodide	acc. to homo-cysteine
	mg.	%	%	%	% of total-S	% of calculated	
Methionine	2.492	96.1	—	—	—	96.1	—
Methionine	6.363						
Cystine	9.262						
Hemin + some other amino acids . . .	12.50	95.5	82.8	117.7	—	109.8	105.3
Methionine	6.528						
Cystine	10.650	93.0	81.4	106.6	2.39	104.5	100.7
Hemin	12.80						

be f = 1,054 and f = 1,217. As may be seen, the cysteine titration gives in both cases too high and varying values. KASSEL and BRAND state that hematin, in contradistinction to inorganic iron, interferes with both the cysteine and the homocysteine titrations, so that these determinations cannot be carried out with e. g. hemoglobin. The cysteine determinations in cytochrome must therefore be regarded as uncertain, though they are able to give some indication in connection with the estimation of the sulphur distribution.

Three determinations were carried out with pure cytochrome (Fe-content 0.43 %; S-content 1.43 %) and the results have been assembled in table 2.

The methionine determinations give results that are fairly close to two molecules of methionine per molecule of cytochrome. The theoretical figure for two molecules (M cytochrome = 13,000) is 2.29 %, while the analyses (according to the corrected values) gave an average of 2.57 %.

For two molecules of cysteine the calculated percentual content is 1.86 % and for three molecules 2.79 %, while the mean figure from the analyses was 2.40 %. Judging from the control-determinations, this value is too high, so that two molecules of cysteine per molecule of cytochrome must be considered more probable.

Table 2.

Cytochrome c weighed amount	Methionine acc. to methyl iodide found	Methionine acc. to methyl iodide corr. f = 1.054	Methionine acc. to homocysteine found	Methionine acc. to homocysteine corr. f = 1.217	Cystine + cysteine found[1]	S found as H_2S & SO_2	Total-S found[2] acc. to methyl iodide	Total-S found[2] acc. to homocysteine
mg	%	%	%	%	%	% of total-S	% of calculated	
245	2.39	2.52	—	—	—	2.94	—	—
276	2.42	2.55	2.28	2.77	2.57	2.59	86.9	84.8
233	2.48	2.61	1.99	2.42	2.22	2.34	83.4	75.9

Inorganic sulphate does not occur in the cytochrome preparation; for SO_4 is reduced quantitatively to H_2S or SO_2, and it would thus be recognized by a higher value on the titration of these.

If one splits cytochrome c with HBr-glacial acetic acid, hematoporphyrin (HILL and KEILIN 1930) is formed, in which connection the cysteine-remains in porphyrin c are split off. Hydrolysis with strong hydriodic acid at 150° has in all probability the same effect, and the two molecules of cysteine that have been found thus belong to the porphyrin. Of the total sulphur an average of 82.5 % was recovered, which corresponds to about five atoms of sulphur per molecule. There thus remains a certain amount of sulphur of unknown nature — one, or more likely (considering the too high cysteine-values) two atoms to give an account of.

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[1] Calculated as cysteine.
[2] Calculated with the uncorrected values; a small amount of methylmercaptan-S must be taken into account (a few % of methionine-S).

From the Physiological Institute, University of Uppsala, Sweden.

The Oil/Water Interface, with and without Monomolecular Film, as a Model of the Living Cell Membrane.[1]

By

STIG SJÖLIN.

(Received 21 August 1942.)

The permeability relationships in living tissues are complex. The cell membrane certainly plays a decisive rôle in ionic migration, ionic partition etc., but attempts to investigate the membrane mechanisms *in vivo* have hitherto not been particularly fruitful. According to the now generally approved conception, cell membranes are built up of lipoids and proteins in layers of several molecules thickness (FRICKE, DANIELLI). The outermost of these layers may conceivably be regularly oriented just as are artificially prepared interfacial monolayers. It has been considered likely that such interfacial films, built up of lipoids or proteins, are analogous to living cell membranes.

RIDEAL, LANGMUIR and LANGMUIR, SERRA and BRISCOE have demonstrated that a monomolecular film in the air/water interface can decrease the velocity of penetration through that interface. Conditions in an oil/water interface appear, however, to resemble those in biological interfaces more closely and are therefore of special interest in this connection. It appears that no quantitative investigations of interfacial films have previously been carried out from this point of view.

The intention of the present research was to ascertain to what degree the velocities of penetration of an oil/water interface, with and without monomolecular film, correspond to those of a living cell membrane. As non-aqueous or oil phase, corresponding

[1] The experimental work described in this paper was carried out in the Institute of Medical Chemistry, University of Uppsala.

to lipoids and proteins in the living cell, benzene was chosen. The permeating substance was salicylic acid, which is soluble in both benzene and water. The velocity of transition of salicylic acid from the water to the benzene phase, and the possibility of influencing the velocity of transition with monomolecular films interposed in the interface were investigated.

Method.

The apparatus is shown in Fig. 1. The system was initially composed of 500 ml. benzene and 500 ml. concentrated aqueous salicylic acid.

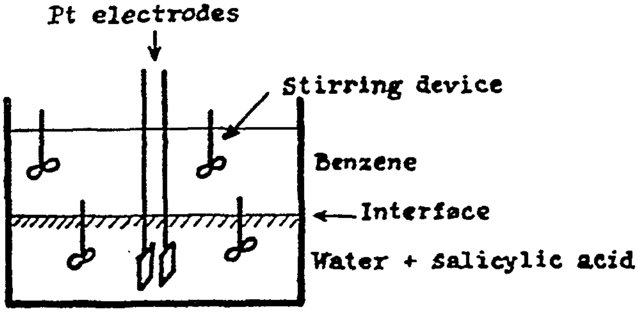

Fig. 1.

The area of the interface was 160 cm². Salicylic acid concentration changes in the aqueous phase were taken as a measure of the velocity of penetration. These changes were followed by measurements of the specific electric resistance of the aqueous solution, made with the help of a Wheatstone's Bridge (General Radio Company) calibrated against salicylic acid solutions of known concentration.

The temperature was kept constant, with a special thermostatic device, at 24—25° with a variation of $\pm$ 0.5° C.

Both phases were stirred so effectively that small undissolved particles could be observed moving with great rapidity in the immediate neighbourhood of the interface. The stirring, however, was never so violent as to cause visible changes in the interface such as vortex formation. The film stabilities were checked by repeated measurements of the interfacial tension. In no case did the stirring disturb the film.

Films of gliadin, lecithin and sodium cetyl sulphate were experimented with. The gliadin (in 60 % ethyl alcohol) and the lecithin (in 60 % isopropyl alcohol) were injected into the interface by means of an "Agla" micrometer syringe according to the method which has been described by ALEXANDER and TEORELL. The sodium cetyl sulphate solution was introduced into the aqueous phase. The lecithin film was found unsuitable for the purpose, since it showed a peculiar reactivity with salicylic acid. This phenomenon will form the subject of more detailed investigation. Another form of interfacial action was

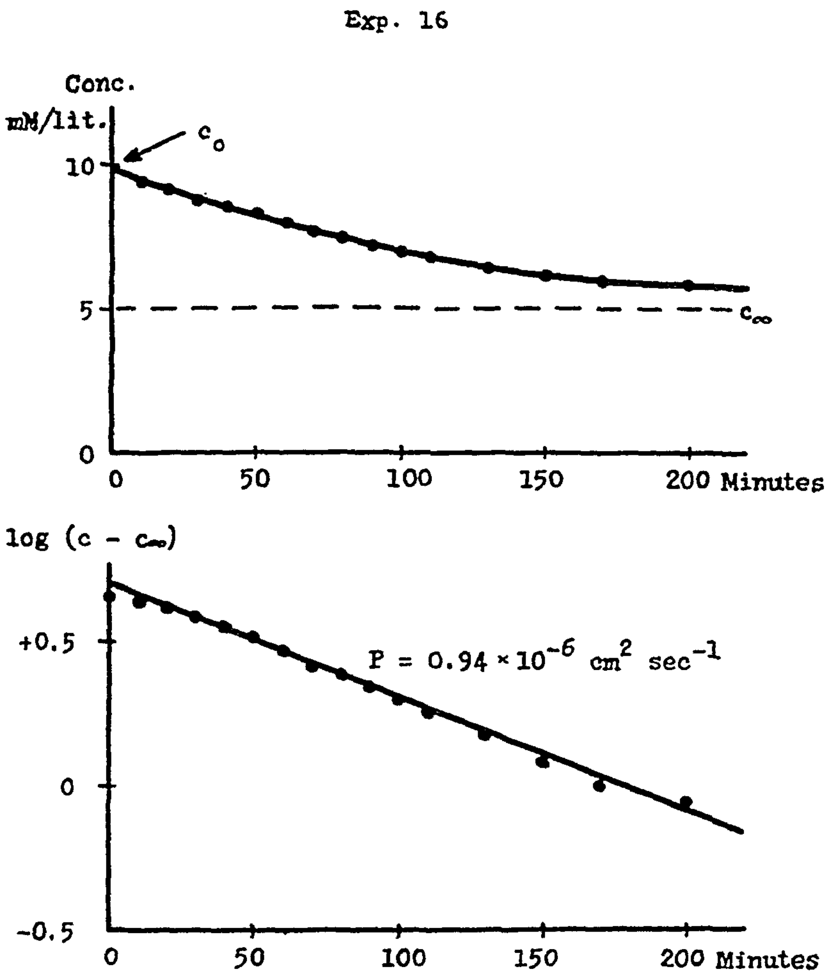

Fig. 2.

observed when the benzene phase was half saturated with lecithin, and gliadin was injected into the interface.

The interfacial tensions were determined by means of the Du Noüy Interfacial Tensiometer (Central Scientific Co.). The interfacial tensions (IT) and the film pressures (F) are given in dynes per cm.

Results.

Both with and without a film in the interface, the approach to the partition equilibrium approximately followed a simple exponential curve, which may be seen from the approximate straigthness of the logarithmic curves (Fig. 2 and 3). The ordinates give the concentrations in millimoles per litre of salicylic acid

It will first be considered whether the penetration velocity is conditioned by pure diffusion processes. To this end we may assume that the penetration of salicylic acid from water to benzene is not influenced by the phase boundary, but by a pure process of diffusion through the convection-free layers on both sides of the interface. From this assumption we may calculate the thickness of the layers. In the penetration constant P the membrane thickness H is not taken into account. The diffusion constant D, however, is calculated for a membrane thickness of 1 cm. If P is to be expressed in terms of D, H must be included in the form D = PH. D for salicylic acid in both water and benzene is about 10^{-5} cm^2 sec^{-1} (International Critical Tables). If the present case is a matter of simple diffusion, Experiment 16 gives the value:

$$H = \frac{10^{-5}}{0.94 \times 10^{-6}} = 10.6 \text{ cm.}$$

and Experiment 19 with the most violent possible stirring:

$$H = \frac{10^{-5}}{3.5 \times 10^{-6}} = 2.8 \text{ cm.}$$

The convection-free layers, however, cannot possibly be so thick. The stirring was always rapid in the neighbourhood of the interface. Experimental investigations by SCHULMAN and TEORELL have shown that the unstirred layers around a cellophane membrane are of about 2×0.003 cm. thickness. There is no resaon to assume that the unstirred layers in the present case should be appreciably thicker. *The penetration velocity therefore cannot be solely conditioned by diffusion.* A quantitative estimate of the part played by the unstirred layers in the penetration of salicylic acid may be obtained by calculating P for a membrane of 2×0.003 cm. thickness, when $D = 10^{-5}$ cm^2 sec^{-1}. Here $P = \frac{10^{-5}}{6 \times 10^{-3}} = 1.66 \cdot 10^{-3}$ cm^2 sec^{-1}. The P found experimentally is 10^{-6} cm^2 sec^{-1}. The discrepancy is therefore about thousandfold.

Obviously the penetration velocity is determined principally by the second alternative given above, namely de resistance offered by the "jump" of the molecules from water to benzene. Actually the salicylic acid molecules must overcome a considerable "energy barrier" (cf. DANIELLI) which thus provides a hindrance to the penetration.

The interfacial films employed did not influence the penetration velocity, which is somewhat surprising in view of the fact that the free interfacial area available for penetration is considerably reduced by the film. In Experiment 12, where sodium cetyl sulphate was the film-forming substance, the film pressure F was 20.0 dynes/cm, at which pressure, according to the curves obtained by ALEXANDER and TEORELL, each molecule has an area of 80Å² at its disposal. Of this area, the sodium cetyl sulphate molecule itself occupies at least 18.6Å², which is the cross-sectional area of the molecule. This implies a blocking of the free penetration area by at least one quarter. Such a figure cannot be given for gliadin since the form and orientation of the molecules are not known. The blocking, however, is certainly at least as great. It has been stated above that the conditions in the phase boundary itself are probably the real determining factors for the penetration velocity in this system. It might therefore be expected that the reduction of available space at the interface would influence the penetration velocity. Since this effect on the part of interfacial films could not be established, there remains the possible explanation that the phase boundary, despite the addition of the film, has not changed its behaviour with regard to the penetrating salicylic acid. It is possible that salicylic acid molecules are dissolved in the hydrophobic parts of the interfacial film as readily as in benzene. In other words, the film-forming molecules may not appreciably influence the energy barrier in the benzene/water interface. It should be recalled that SEBBA and BRISCOE found that certain films do not appreciably influence the velocity of evaporation of water molecules from water to air. It might say that the water molecules were "dissolved" by the film.

For biological membranes (red blood corpuscles, sea urchin eggs), various workers (WILBRANDT) have found penetration constants varying between 10^{-5} and 10^{-9}cm²sec⁻¹. The velocity constants obtained in the model experiment above, 10^{-6}cm²sec^{-1}, are therefore quite comparable with the biological values. An attempt to a theoretical calculation of the "penetration constants" for an oil/water interface is to be found in DANIELLI's paper. He also finds good agreement between these and the biological values by assuming that the membrane has a viscosity 10^5 times that of water. There is therefore reason to consider, and to attempt to explain the permeability relations of living cell membranes in

respect of the results of the model experiments. In the latter it is the "jump" from water to benzene which determines the penetration velocity. In living membranes, the source of the resistance to penetration should not be sought merely in pore width, pore length etc. In the case of substances *soluble* in the membrane, it should also be sought in the actual passage across the phase boundary *between* the water and lipoid etc. phases. It appears desirable that these partition kinetics should be more closely studied, with special regard to their biological significance.

Summary.

(1) An oil/water interface (benzene/salicylic acid solution) has been employed as a model of a cell membrane.

(2) The velocity of penetration of salicylic acid from the aqueous into the non-aqueous phase, and the influence of interposed monomolecular interfacial films (gliadin, sodium cetyl sulphate) on the penetration velocity have been investigated.

(3) The penetration constants found were of the order of $10^{-6} cm^2 sec^{-1}$. An effect of interfacial films on the penetration could not be demonstrated.

(4) The reasons for the failure of the interfacial films to affect the penetration are discussed. The influence of the unstirred layers around the phase boundary is found to be negligible. It is concluded that the penetration velocity is principally determined by an "energy barrier" situated in the phase boundary, which must be overcome by the penetrating molecules.

(5) The order of magnitude of the penetration constants found in model systems is the same as that observed for living cell membranes. The kinetics of penetration through the latter may therefore be considered as determined by the conditions at the phase boundary as well as by the pore dimensions etc.

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Herstellung und Eigenschaften von Substanz P.

Von

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Eingegangen am 24. August 1942.

Die vorläufig als Substanz P bezeichnete glattmuskelerregende Substanz aus Darm und Gehirn (EULER und GADDUM, 1931) wurde später als ein Eiweisskörper erkannt (EULER, 1936). Sie bewirkt bei Kaninchen eine vorübergehende Senkung des Blutdruckes und erregt die Motilität von glattmuskeligen Organen in vitro (EULER, 1936, BJURSTEDT, EULER und GERNANDT, 1939) und in vivo (GERNANDT, 1941).

Herstellung. Frischer Pferdedarm wird gewaschen, zerkleinert und mit 2 Volumina bis pH 4 mit H_2SO_4 angesäuertem Wasser 2-mal bei 100° extrahiert. Das Filtrat wird in vacuo soweit eingeengt, dass 1 ml 20 g Darm entspricht. Das Extrakt wird mit NaOH bis pH 8 und mit $2^1/_2$ Volumina Alkohol versetzt. Die Fällung wird nach 24 Stunden im Eisschrank abfiltriert, wieder in dem halben ursprünglichen Volum Wasser suspendiert und nochmals mit $2^1/_2$ Volumina Alkohol versetzt. Die vereinigten Filtrate werden mit Essigsäure neutralisiert und soweit eingeengt, dass 1 ml 50 g Darm entspricht. Der Fällungsrückstand wird verworfen. Das Konzentrat wird dann mit Essigsäure bis pH 4 versetzt, zentrifugiert und die Fällung mit Wasser gewaschen. Die Fällung wird verworfen und die klare braune Lösung mit Ammonsulfat bis 0.6 Sättigung versetzt und über Nacht im Eisschrank gelassen. Die ausgeschiedene Substanz wird abfiltriert, abgepresst, wieder in Wasser gelöst und nochmals mit Ammonsulfat bis Sättigung bei pH 4 ausgesalzt, abfiltriert, abgepresst und getrocknet. Von 14.5 kg Darm wurden in einer Präparation 33,000 Einheiten[1] in 6.56 g Trockenpräparat gewonnen (0.2 mg pro E), in einer andern Präparation von 10 kg Darm 32,000 E. in 9.56 g (0.3 mg pro E.).

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[1] Die Einheit entspricht etwa der Wirkung von 2—4 Schwellendosen am isolierten Jejunumstück des Kaninchens in einem 30 ml Bad.

Auch aus Hirn konnte die wirksame Substanz durch Aussalzen in derselben Weise gewonnen werden. (BJURSTEDT, EULER und GERNANDT, 1940).

Zwecks weiterer Reinigung wurde eine konzentrierte Lösung des Präparates (15—20 %) mit NaOH bis pH etwa 8 versetzt und mit 4 Volumina 96 % Alkohol versetzt. Dabei wurden inerte Proteinstoffe nebst Natriumsulfat nach Abtreibung des Ammoniaks in vacuo ausgefällt. Die besten Präparate enthielten nach dieser Reinigung 1 E in 0.17 mg.

Für die weitere Reinigung wurde Fällung mit Pikrinsäure vorgenommen. Eine wässerige Lösung der Substanz P wurde mit gesättigter alkoholischer Pikrinsäurelösung vollständig gefällt. Bei einer Alkoholkonzentration von 25 % oder weniger wurde Substanz P fast vollständig gefällt. Durch Auslaugen der Fällung mit Eisessig wurde eine weitere Reinigung bewerkstelligt, wobei inerte Proteinstoffe ungelöst blieben.

Die Pikrate waren in etwa 50—70 % Alkohol am besten löslich. Durch Auslaugen der Pikrate mit Methylalkohol wurden weiter gereinigte Präparate mit 1E in 0.13 mg gewonnen.

Wirksame Pikrate wurden dekomponiert (Schwefelsäure, Äther) und die zurückgewonnene Substanz biologisch geprüft, wobei die Wirksamkeit unverändert war.

Weitere Versuche die aktive Substanz mit Silbersalzen und Reineckesäure zu reinigen ergaben unbefriedigende Resultate, da die volle Aktivität weder aus der Fällung noch aus dem Filtrat zurückerhalten wurde. Die Substanz lässt sich bei saurer Reaktion mit Azeton aus alkoholischer oder wässeriger Lösung ausfällen. Es liess sich aber eine teilweise Inaktivierung in manchen von diesen Versuchen feststellen.

Kataphoreseversuche. Dekomponierte Pikrate gelangten in Azetatpuffer bei pH 5.6, 6.43 und 7.09 in dem Apparat von Tiselius während 10—12 Stunden zur Kataphorese. Bei den beiden ersteren pH-Werten wanderte die aktive Substanz nach der Kathode, bei pH 7.09 nach der Anode. Die wirksame Substanz verhält sich somit wie ein amphoterer Körper, dessen isoelektrischer Punkt zwischen pH 6.43 und 7.09 liegt, was mit den bei Fällungsversuchen und früheren Elektrodialyseversuchen (EULER, 1936) gemachten Befunden in Einklang steht, laut denen die Substanz P sich als ein schwach basischer dialysabler Proteinkörper (Polypeptid) verhält.

Zusammenfassung.

Eine Methode zur Herstellung gereinigter Präparate von Substanz P aus Pferdedarm wird beschrieben. Die besten Präparate zeigten ohne weitere Reinigung eine Wirkung von 1 E. in 0.2 mg Substanz.

Die Substanz lässt sich als Pikrat ausfällen. Gereinigte Pikrate zeigten eine beste Wirksamkeit von 1 E in 0.13 mg.

Kataphoreseversuche ergaben, dass die Substanz noch bei pH 6.4 kathodisch und bei pH 7.1 anodisch wandert.

Diese Untersuchung wurde durch Mittel der Stiftung »Therese och Johan Anderssons minne» unterstützt. Für wertvolle Hilfe spreche ich Frl. M. Nyborg und cand. med. G. Engstedt meinen besten Dank aus. A. B. Astra, Södertälje, hat gütigst Rohextrakte zur Verfügung gestellt, wofür ich hier danken möchte.

Litteratur.

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Determination of the Red Corpuscle Content.

By

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Some time ago, a method of determination of the red corpuscle content, the "erythron", was described (HAHN and HEVESY 1940). Corpuscles containing labelled phosphorus compounds are introduced into the circulation of the rabbit, for example, and the labelled phosphatide content, or the labelled acid soluble phosphorus content of the corpuscles of samples secured after the lapse of few minutes, is compared with the corresponding content of the corpuscles injected. Such a comparison indicates to what extent the labelled corpuscles introduced into the circulation are diluted by the corpuscles of the circulation, and it permits thus the calculation of the erythron of the rabbit.

In the present note, a simplified form of the above mentioned method is described. The modified method is based on the comparison of the total labelled phosphorus content of the corpuscles injected with the total labelled phosphorus content of the corpuscles secured after the injection took place. The corpuscles are labelled *in vitro*. A blood sample of the rabbit is shaken in the thermostat for 1—2 hours in the presence of labelled sodium phosphate. By this procedure the corpuscles get labelled. The blood containing the labelled corpuscles is then reintroduced into the circulation of the rabbit. This modification of the previously described procedure was worked out in view of a possible clinical application of the method.

Labelling of the Corpuscles.

About 20 cm^3 of blood are removed from the rabbit, placed in a flask the walls of which are coated with paraffin, labelled phosphate of negligible weight (see below) is added, and the blood is gently shaken in a thermostat for 2 hours at 37°.[1] 10 cm^3 of the blood are then reintroduced into the circulation of the rabbit. From the remaining blood, two standard samples, each of them weighing about 3 gm, are prepared.

3 minutes after the injection of the labelled blood into the jugular vein, a few cm^3 of blood are taken from the carotis. Further samples are taken at later times. The heparinized blood samples are centrifuged, the corpuscles are weighed and brought into solution by wet ashing; subsequently, 80 mg of sodium phosphate are added, and the phosphate content of the solution is precipitated as ammonium magnesium phosphate. The standard samples are treated in a similar way. In view of the much larger activity of the standard samples, only $^1/_{20}$ of the solution obtained after ashing the sample is precipitated.

Let us denote the amount of corpuscles injected into the rabbit by A, the ratio of the activity of 1 gm corpuscles of the blood injected and of the activity of 1 gm corpuscles secured from the circulation after the injection as B, then the total amount of the corpuscles present in the circulation (X) is given by

$$X = A \cdot B.$$

In some of our experiments, we used ^{32}P prepared from carbondisulfide which was previously irradiated by a neutron beam. Should some of the ^{32}P present in the solution obtained by the extraction process be adsorbed by colloidal particles or be present in the solution in another not properly dissolved state, this part of the ^{32}P will be found after centrifuging the standard blood sample in the fraction containing the erythrocytes, while in the circulation this part may be taken up by the reticulo-endothelial cells. In order to avoid a possible error due to such an effect, we did not add the ^{32}P directly to the blood to be investigated but to a small blood sample which was centrifuged at once. The plasma of the last mentioned sample containing ^{32}P was added to the blood to be shaken in the thermostat.

An alternative method is the following one. Labelled phosphate is administered to a rabbit, the blood of which thus becomes labelled. Few cm^3 of the labelled blood are introduced into the circulation of another rabbit after the lapse of several hours. By this procedure the activation in vitro can be avoided.

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[1] This high temperature was chosen to accelerate the penetration of labelled phosphate into the corpuscles.

Is the Label of the Corpuscles Properly Conserved?

The method described above is based upon the assumption that the ^{32}P introduced into the corpuscles while the blood containing labelled phosphate is shaken in the thermostat is not given off during the experiment. One might expect that after the introduction of active blood into the inactive circulation ^{32}P will leave the corpuscles and get replaced by ^{31}P atoms of the plasma. Such a process, if taking place at a sufficient rate, would clearly frustrate the application of the method. Since, however, a mixing of the blood introduced into the circulation of the rabbit with the circulating blood does not last more than some minutes or less, the blood sample can be secured, for example 3, 5, 7, and 9 minutes after the injection took place.

The loss of activity of the corpuscles in experiments in which active corpuscles were shaken in the thermostat with inactive plasma for 12 min. was found to amount to 1.5 per cent, only. (Cf. also HAHN and HEVESY 1940). That a possible loss of the labelling ^{32}P by the corpuscles does not influence our results is also seen in Table 1, in which the average of the activity of 1 gm of corpuscles secured from 12 rabbits after 3, 5, 7, 9, and 15 min., respectively, is stated. The values obtained after 5, 7, and 9 min. do not differ from each other within the error of experiments ($\pm$ 5 per cent). The value obtained after the lapse of 15 min. possibly indicates a slight loss of ^{32}P by the labelled corpuscles.

The fact that, within a time amply sufficient to carry out determination of the erythron the label of the corpuscles remains conserved is due partly to the comparatively slow rate of passage of the phosphate ions through the corpuscle wall, and partly to the fact that the easily renewable (activated) acid soluble P content of the corpuscles is much higher than the corresponding fraction of the plasma. The major part of the ^{32}P which entered the corpuscles while the blood was shaken in the thermostat is present as easily exchangeable organic acid soluble P. Now, the ^{32}P atoms have the same chance to leave the corpuscles as have the ^{31}P atoms present in the same state. The ratio of ^{32}P and ^{31}P atoms in the corpuscles is, however, much smaller than the corresponding ratio in the plasma. Correspondingly, the chance of a ^{32}P atom to leave the corpuscles is comparatively small. The corpuscles of 100 gm of

rabbit blood contain about 20 mg easily renewable acid soluble P atoms, while the plasma contains only about 2 mg P atoms; the ^{32}P content of 1 gm corpuscles in the labelled blood injected is about the same as the ^{32}P content of 1 gm plasma. The ^{32}P atoms have thus a much greater chance to enter the corpuscles than to migrate in the opposite direction. Of course, if an exchange equilibrium of ^{32}P between plasma and corpuscles is reached, this statement is no longer valid, but — as follows from the above data — after shaking the blood at 37° for 2 hours, the $^{32}P : {}^{31}P$ ratio of the plasma is still about 10 times the corresponding ratio in the corpuscles.

Table 1.

Activity of 1 gm corpuscles secured from different rabbits at different times after the injection of labelled blood (corpuscles).

Time in minutes	3	5	7	9	15	Rabbit
Activity (taking the activity found after the lapse of 3 minutes to be = 100)	100	106		104		B. 2
	100	102	95		96	B. 3
	100	104	91		89	B. 6
	100	105		106	97	B. 7
	100	107		105	92	B. 8
	100	95	96	110		B. 9
	100		102	99		B. 10
	100		100	96		B. 11
	100	93	95	95		C. 1
	100	100	97			C. 2
		100	97	93		C. 3
	100	104		95		C. 4
Average activity	1100 : 11 = 100	1016 : 10 = 102	773 : 8 — 97	903 : 9 = 100	374 : 4 = 94	

Errors due to the Activity of the Plasma.

We do not inject active corpuscles but active blood into the circulation of the rabbit. The plasma of the rabbit becoming thus active, some active phosphate will penetrate into the corpuscles in the course of the experiment, increasing thus the ^{32}P content of the corpuscles. Such a process may entail a source of error. If, besides the corpuscles injected, labelled corpuscles are formed in the circulation, the value calculated for the erythron from the dilution figures will clearly be found too low. In order to estimate the error due to the above mentioned process the following ex-

periment was carried out. Active plasma is injected into the blood of a rabbit and blood samples are secured at different times. The corpuscles are separated and their activity is determined. The figures obtained (cf. Table 2) show what percentage of the plasma activity enters the corpuscles during the experiment (3 to 12 min.), similar values being obtained in further experiments. In our erythron determination, the activity of 1 g corpuscles injected was about the same as the activity of 1 g plasma injected, i. e. in the relative units of Table 2 = 1,000. Therefore the figures of the 4th column of Table 2 give almost exactly the percentage error of the erythron determination due to the penetration of ^{32}P from the plasma into the corpuscles in the circulation of the rabbit.

It is of interest to compare the rate of penetration of ^{32}P from the plasma into the corpuscles in experiments in vitro with the figures obtained in the above described experiment in vivo.

Experiment in vitro.

Inactive blood was brought to 37° in the thermostat, active plasma of negligible weight was then added and the activity of the corpuscles was determined at different times. After the lapse of 3, 6, and 12 min., respectively, the corpuscles were found to contain 5.4 %, 8.8 %, and 12.9 % of the activity of the plasma. These figures were corrected for the activity due to adhesion of active plasma to the corpuscles which was found to make out 2 % of the plasma activity. In experiments in vitro in which the plasma activity does not much change during the experiment the activity penetrating into the corpuscles during 3 to 12 min. is thus quite appreciable. In experiments in vivo, however, the plasma activity rapidly decreasing after the injection of the active blood, the amount of ^{32}P penetrating into the corpuscles is much smaller (cf. Table 2). If the plasma activity would disappear with the same speed in experiments in vitro as in experiments in vivo we would obtain the figures seen in the 5th column of Table 2.

The following example shows how column 5 of Table 2 was calculated. Calculation of the value obtained after 3 min. The average activity of 1 g plasma during the 3 first minutes can be estimated to be 500. As, after 3 min., in the experiment in vitro

1 g corpuscles was found to contain 5.4 % of the activity of 1 g plasma, the figure registered in column 5 works out to be 2.7. The average activity between 3 and 6, and between 6 and 12 min., respectively, is estimated to be 200 and 130, respectively.

When comparing the activity of the corpuscles with the activity of the plasma, we can calculate the increase in activity of the corpuscles and compare this activity increase with the activity of the corpuscles which we inject in our usual experiments in which blood is shaken with active phosphate in the thermostat before the injection.

Table 2.

Penetration of ^{32}P of the plasma into the corpuscles after injecting labelled plasma into the rabbit.

Time in min.	Activity of 1 g corpuscle	Activity of 1 g plasma	Percentage of the plasma injected present in the corpuscles	
			found	calculated from experiments in vitro
0	0	1000	0	0
3	26	260	2.6	2.7
6	33	158	3.3	3.4
12	40	99	4.0	3.9

Adhesion of Active Plasma to the Corpuscles.

The activity of the plasma may also influence the results obtained in another way as mentioned above. Centrifuging of blood does not lead to corpuscles entirely free of plasma. After centrifuging blood for 25 min., in a centrifuge making 5,000 revolutions per minute we find the corpuscles to contain 2 % plasma (cf. also HAHN and HEVESY 1942). In 1 g corpuscles prepared from the blood to be injected, we shall therefore find only 0.98 g corpuscles, the remainder being composed of plasma. In the blood to be injected, the activity of 1 g corpuscles is about equal to the activity of 1 g plasma, and the error due to the presence of plasma in the blood injected thus can be disregarded. However, other conditions prevail in the blood samples secured from the rabbits at different times. In the last mentioned samples the adhering plasma is much less active than the corpuscles to which the plasma adhers. The activity of 1 g of the corpuscles secured from the

active circulation is thus not strictly comparable with the activity of 1 g of the corpuscles injected. Knowing the activity of the plasma at different times (cf. Table 2) we can correct for this discrepancy. The erythron value calculated without taking regard to the above mentioned source of error will be 1.5 %, 1.7 % and 1.8 % respectively too high. We could eliminate the above mentioned sources of error due to the activity of the plasma by removing the active plasma and replacing it by inactive one. However, this step would complicate the procedure.

Not only, however, remains the error due to the adherence of the plasma to the centrifuged corpuscles within the errors of the experiment, it is in fact to a large extent compensated by the error due to the penetration of active phosphate from the plasma into the corpuscles during the few minutes which elapse between the injection of the active blood and the collection of the samples.

An estimate of the three errors of the experiment, — viz. 1) adhesion of plasma to the centrifuged corpuscles, 2) penetration of ^{32}P from the plasma into the corpuscles in the circulation of the rabbit (error due to the fact that we do not inject labelled corpuscles but labelled blood), 3) loss of ^{32}P by the corpuscles during the experiment — is seen in Table 3. After the lapse of 3 min., the plasma adhering to the corpuscles is thus less active (per g) than the corpuscles to which it adhers. Since we determine the weight of the corpuscles + adhering plasma and the adhering plasma (per g) is only $^1/_4$ as active as the corpuscles, we find the activity of the corpuscles too low, the error being $2 - 2 \cdot {}^1/_4 = 1.5$ per cent. Correspondingly, after 5 and 7 min., respectively, we commit an error of about 1.7 per cent.

Table 3.

Estimate of different errors of experiment in the determination of the erythron.

Time in min.	Percentage error due to adherence of the plasma to the corpuscles	Percentage error due to intrusion of ^{32}P of the plasma into the corpuscles	Percentage error due to the loss of ^{32}P by the corpuscles
3	+ 1.5	— 2.7	+ 0.5
6	+ 1.6	— 3.4	+ 1
12	+ 1.8	— 3.9	+ 1.5

Results.

As seen in Table 4, the corpuscle content of the rabbit per kg of fresh weight varied between 20.5 and 26.5 gm. It is difficult to decide whether the erythrocyte reserve present in the spleen and some other organs interchanges with the erythrocytes of the circulating blood and, correspondingly, to what extent it participates in the dilution of the labelled corpuscles. Since our experiments were carried out with narcotized rabbits and, as shown by BARCROFT (1934), in ether narcosis the blood reserves of the body are to a very large extent released, our results are independent of the above mentioned source of error and indicate the total erythrocyte content of the rabbits investigated.

Table 4.

Rabbit	Weight in gm	Weight of corpuscles injected	Activity per gm corpuscles injected	Activity per gm corpuscle sample[1]	Total corpuscle content (erythron) in gm	Corpuscle content in gm per kg rabbit weight
B. 2 . .	3050	3.40	2006	108	63.1	20.7
B. 3 . .	2290	2.66	2470	140	46.9	20.5
B. 6 . .	2470	3.98	1660	115	57.5	23.3
B. 7 . .	2550	3.71	2160	153	52.4	20.6
B. 8 . .	3200	3.38	2040	83.3	82.9	25.9
B. 9 . .	1990	3.06	2450	181	41.4	20.8
B. 10 . .	3160	3.10	2102	99.7	65.4	20.7
B. 11[2] .	2010	3.23	2160	240	29.1	14.5

Radio-Phosphorus and Radio-Iron as Indicators.

HAHN, BALFOUR and alia (1941) determined the corpuscle volume of the dog, using radio-iron as an indicator. Within a few days after administration of radio-iron to dogs, most of the radio-iron present in the body is concentrated in the erythrocytes as a constituent of the hemoglobin molecules. Such erythrocytes labelled by the presence of radio-iron were used in the same way to determine the erythron of the dogs as corpuscles labelled by the presence of radio-phosphorus were applied by HAHN and HEVESY (1940) and by the present writers to determine the

[1] Average of samples secured between 3 and 9 min. (comp. Table 1).
[2] Anemia following previous operation. Hematocrit value = 22.5.

erythron of the rabbit. In the determination of the erythron, the radio-phosphorus method has the following advantages. Blood samples can be activated *in vitro*, while such a procedure cannot be carried out when using radio-iron. Corpuscles containing labelled hemoglobin can only be obtained in experiments *in vivo*. Furthermore, radio-phosphorus is very much easier to procure than radio-iron, since the preparation of radio-iron in sufficient quantities requires powerful tools in contrast to the preparation of radio-phosphorus.

Summary.

A simplification of the method of determination of the red corpuscle content (the erythron) previously communicated is described.

A blood sample taken from a rabbit is shaken in the thermostat at 37° for about 2 hours in the presence of labelled sodium phosphate and, then, it is reintroduced into the circulation of the rabbit. A comparison of the total labelled phosphorus content (radioactivity) of the corpuscles injected with the total labelled phosphorus content of corpuscle samples secured few minutes after the injection took place leads to the value of the erythron.

The corpuscle content of the rabbits investigated varied between 20.5 and 26.5 gm per kg of rabbit weight.

The authors wish to express their heartiest thanks to Professor NIELS BOHR for numerous facilities kindly put at their disposal.

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FROM THE PHARMACOLOGICAL DEPARTMENT OF THE CAROLINE INSTITUTE
AND THE MEDICINAL CLINIC OF THE SERAPHIMER HOSPITAL, STOCKHOLM.

THE OXYGEN DEFICIT OF ARTERIAL BLOOD CAUSED BY NON-VENTILATING PARTS OF THE LUNG

BY

SVEN M. BERGGREN

To my parents

CONTENTS.

PREFACE.

During the whole time I have been occupied with this work, I have had the great advantage of having had access to the expert knowledge that Professor Göran Liljestrand possesses in the physiology of blood-gases; he has been of invaluable help for me in solving the many problems that have arisen in connection with the planning of the work and the drawing up of the method. For all the assistance he has given me and for the favourable and pleasant conditions under which I have worked in his department I wish to express my heartfelt thanks.

The greater part of the clinical material I have obtained from the Medicinal Clinic of the Seraphimer Hospital, and I am greatly indebted to Professor Anders Kristenson, the Head, for the interest he has taken in my experiments, for all valuable criticism and for all the encouragement he has always given me.

I wish to thank Professor Josua Tillgren and Professor Gustaf Söderlund as well as Docent Clarence Crafoord and Docent Oscar Schuberth for so kindly handing over occasional cases.

While working at the Pharmacological Department and at the Seraphimer Hospital I have been repeatedly obliged to apply to their staffs for assistance, and the helpfulness they have always shown me has been of the greatest value.

Many of the problems belonging to this subject have been exhaustively discussed with colleagues of my own age. The valuable opinions that have been expressed during these discussions as well as the stimulating comradeship will always stand out as one of the most pleasant memories from this time.

For the translation I wish to thank Miss Dorothy Ferris for all the interest and care she has taken.

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Stockholm, April 1942.

Sven M. Berggren.

INTRODUCTION TO PROBLEMS.

The Oxygen Tension Equilibrium between Alveolar Air and Arterial Blood.

After the first investigations by Hürter (1912) on the gases in the arterial blood of man, a considerable number of investigations on this subject have been published (Stadie 1919, Harrop 1919, Le Blanc 1922, Meakins and Davies 1925, and others), and in several diseases a considerable lowering of the oxygen content of the arterial blood in comparison with normal cases has been established as a sign that the absorption of oxygen in the lungs is seriously deranged. In their synopsis on cyanosis Lundsgaard and Van Slyke (1923) have given a theoretical analysis of the different factors causing an arterial anoxemia. In spite of the number of arterial blood-gas analyses published, there are few that make it possible to form a quantitative conception of the relative importance of these factors in each particular case.

The factors to be considered are as follows:

1. The composition of the alveolar air.
2. The diffusion through the alveolar epithelium.
3. Admixture of venous blood to arterialized blood.

ad 1. In order to be able to retain the oxygen content of the alveolar air at a satisfactory level, a constant supply of air is required by means of breathing movements corresponding to the consumption of oxygen. The defective renewal of the alveolar air can either concern all parts of the lung, e. g. in barbiturate or morphia poisoning or the superficial, ineffective breathing in certain cases of pneumonia, or it may only concern certain parts of the lung, e. g. in the case of emphysema. This irregularity in the ventilation of different parts of the lung has been of considerable importance when discussing its function. According to a number of investigators, it seems probable that in the healthy lung there

are parts that are less ventilated than others (TENDELOO 1902, NIELSEN and SONNE 1932, ROELSEN 1937, and others), but it is not certain whether this irregularity is of any importance to the function. If a reduced ventilation in one part of a lung is compensated by a corresponding reduction of the blood-flow, the composition of the alveolar air will not be different from that in other parts. The fact that fractionated alveolar samples from one and the same respiration show a difference in oxygen content, which is greater between the first samples than between the last, is no proof that these last fractions originate in parts with a deficient ventilation, as has at times been supposed (SONNE 1934). They may just as likely be signs of a diminished blood-flow through the lungs during the latter phase of expiration (KROGH and LINDHARD 1914).

As far as oxygen is concerned, this possible irregularity in the alveolar ventilation is of greater importance than is the case with carbon dioxide. On account of the different modes of combining, the reduced elimination of carbon dioxide within a sphere with hypo-ventilation is compensated by an increased elimination within the hyper-ventilated parts. The deteriorated oxygen absorption in a badly ventilated area, however, cannot be compensated in the hyper-ventilated parts, the oxygenation of the hemoglobin being all but complete already with normal ventilation (HALDANE and PRIESTLEY 1935).

ad 2. The discussion as to whether oxygen passes into the blood by diffusion or by secretion (BOHR, HALDANE) seems to have been decided in favour of the former process after KROGH's enlightening work (1910), in which the oxygen tension was determined by means of a perfect method and was compared with the alveolar oxygen tension. HARTRIDGE (1912—13) has revised HALDANE's experiments on oxygen secretion with the carbon monoxide method but with a more accurate manner for determining the CO-hemoglobin, and he has not been able to prove that the oxygen tension of the blood was above that of the alveolar air, not even during muscular work or at lowered oxygen tension.

The stage of equilibrium to which the diffusion proceeds, cannot, however, be considered as established. The tension difference seems pretty regularly to vary from a few up to 20—30 mm. (KROGH and KROGH 1910, BARCROFT, COOKE et al. 1920, BOCK, DILL et al. 1929, KROETZ 1931, DILL, EDWARDS et al. 1931, KRAMER and SARRE 1935, DILL, CHRISTENSEN et al. 1936, MATTHES et

al. 1936, BRINKMAN and DIRKEN 1940). It seems doubtful, however, whether this tension difference is an expression for an incomplete diffusion equilibrium, especially if consideration is taken to the direct determinations of the diffusion constant by M. KROGH (1914) with BOHR's (1909) carbon monoxide method. She found a diffusion constant (i. e. ml. oxygen which pr mm. tension difference passes from the alveolar air into the blood during 1 min.) in normal subjects ranging between 24 and 43 ml. oxygen. The absorption of oxygen in the lungs largely takes place under a fairly great gradient of tension, while towards the end of the diffusion when the difference of tensions rapidly diminishes, only small quantities diffund. As has been pointed out by BOHR (1909), this depends on the form of the oxy-hemoglobin dissociation curve, from which it appears that the oxygen saturation in the region of almost complete saturation increases only slightly at considerable increase of the tension. Making use of a formula given by BOHR (1909), and calculating the diffusion constant from the final tension difference, Sarre (1935) arrived at a value for the diffusion constant of only 12.5 ml when the tension difference was 0.5 mm. The empiric values given by M. KROGH thus suffice to let the diffusion proceed to a point, where the tension difference is but a fraction of a mm.[1] The explanation for the tension differences found must under such circumstances be sought in some other factor (BARCROFT 1925).

Much seems to indicate that one of these factors is the difficulty of getting a reliable expression of the average oxygen concentration of the alveolar air; this is due to the variations of concentration during the different phases of respiration and to the irregularity in the composition of air from the different parts of the lung. The admixture of blood from the bronchial veins to the fully oxygenated blood from the alveoli can likewise contribute to the difference in oxygen tension between alveolar air and blood (KNIPPING 1935). As DILL, CHRISTENSEN et al. (1936) point out,

[1] According to ROUGHTON (1934) the method of BOHR and KROGH does not give reliable values, because the assumption that the carbon monoxide tension in blood is practically zero does not hold good, the oxygen combining so much more rapidly with the hemoglobin than carbon monoxide. In these circumstances, however, the difference in tension by means of which the diffusion constant has been worked out, would be less than was calculated. The constant would thus be greater than the values found by KROGH. The greater diffusion velocity for oxygen, compared with carbon monoxide as determined by A. KROGH (1919) would raise the values still further. The diffusion constants given by M. KROGH should thus be considered minimum values.

it is therefore incorrect to interpret the existing tension difference as a diffusion gradient.

ad 3. Under normal circumstances there are possibilities for the admixture of venous to fully arterialized blood in the form of anastomoses between bronchial and lung veins (MILLER 1906, 1925, MATHES et al. 1932). Though the exact magnitude of this admixture is unknown, it is probably small. In pathological circumstances, however, the mixing of venous and arterialized blood will prove an important cause of defective oxygen saturation of arterial blood, as e. g. in the case of pneumonia and atelectasis, and above all in cases of congenital heart disease (Morbus coeruleus).

An evaluation of the relative importance of the factors described as causes of a defective arterial oxygen saturation seems to be impossible for the present, owing to the lack of quantitative measurements of these factors. Apart from a few determinations of the diffusion constant of the lung which were carried out by M. KROGH on patients with emphysema and pneumonia, in which cases the diffusion constant was normal, and by HARROP and HEATH (1927) in polycythemia, where a small reduction was found, no direct determinations of this factor have been carried out in pulmonary diseases. The BRAUER-KNIPPING school considers the impaired diffusion to be of paramount importance in arterial oxygen unsaturation (KNIPPING 1935) and for such a state BRAUER has introduced the conception pneumonosis. The difficulty of obtaining a reliable proof of this impaired diffusion is admitted.

The decrease in arterial oxygen saturation by inhaling air with lowered oxygen content is in some cases of heart and lung diseases larger than normal and this test has been used in the study of pulmonary function (KROETZ 1929, DIRKEN and KRAAN 1937, DIRKEN et al. 1942). The last mentioned authors in some cases of bronchitis and pneumothorax found a low oxygen saturation already during the inhalation of normal air but the decrease from this value while inhaling a mixture of low oxygen content was the same as in normal cases. This was considered to indicate the presence of non-ventilated parts of the lung through which blood was flowing, whereas the alveoli in ventilated parts were normal. The observations are few, and the variations in normal cases considerable which greatly invalidates the conception of the authors.

By judging the effect of breathing oxygen some investigators have tried to obtain a qualitative conception of the existence of

an admixture of venous blood. An arterial oxygen unsaturation, which is not relieved by oxygen, proves that circulation is continued through areas cut off from ventilation (MEAKINS and DAVIES 1925). As the oxygen unsaturation, however, in most cases of pneumonia is relieved by oxygen, these authors ascribe the unsaturation to the deficient breathing (MEAKINS and DAVIES 1921).

In the present work a method will be described rendering possible a quantitative determination of the size of the arterial oxygen deficit arising in consequence of the admixture of venous blood to fully arterialized blood, whether this is caused by the blood-flow through a lung area cut off from ventilation, or by blood short-circuited in some other manner between the right and the left side of the heart.

The Blood-Flow through Non-Ventilated Parts of the Lung.

In a number of different ways and with varying results attempts have been made to form some idea of the blood-flow through the lung in different stages of collapse and infiltration.

When perfusing a lung blown up to different volumes, POISEUILLE (1855) found that the perfunded quantity decreased the more the lung was blown up. QUINCKE and PFEIFFER (1871), who effected an expansion of the lung in a more physiological manner, i. e. by negative external pressure, came to a contrary result. In his synopsis of the lung circulation, TIGERSTEDT (1903) writes that it must be considered definitely established that the blood-vessels of the lung expand under inspiration and are narrowed under expiration. In later investigation which CLOETTA (1910—1919) carried out by means of lung plethysmography he considered that the blood-flow is increased in an expiratory position of the lung, but as his results are open to criticism (KROGH and LINDHARD 1914, LE BLANC 1922, CORYLLOS and BIRNBAUM 1929), there seems no reason repudiate TIGERSTEDT's conception.

By extracting the blood-pigment from normal and atelectatic areas of the lung, BRUNS (1909—1914) found a smaller blood content in the atelectatic parts. By determining the number of blood cells in a histological section HANSON and SJÖSTRAND (1935) came to the same result.

The method of injecting a dye or other substances settling in the tissues in proportion to the blood-flow has been made use of for the same problem. By injecting aniline blue and a gelatinous solution of Prussian blue, ROMMELAERE (1881) did not get any filling of the arterial branches or any colouring of the tissue within an atelectatic area, and he considered that the blood-flow is stopped up by the atelectasis. CORPER, SIMON and RENSCH (1920) injected Prussian blue and scarlet-red suspension intravenously and obtained a uniform colouring of the lungs when in a normal state as well as in a condition of pneumothorax, except when this was of long duration, when a reduced colouring resulted. From the illustrations published it seems, however, as if the lung was only reduced in size, without any atelectasis having developed. According to CORYLLOS and BIRNBAUM (1929), the distribution of injected iodized oil and Indian ink showed a reduction in the arterial blood supply and a disappearence or narrowing of capillaries in atelectatic and pneumonic parts of the lung. With a suspension of bismuth MATHES, HOLMAN and REICHERT (1932) obtained the same injection of the arterial system of the lung when it was in a state of atelectasis as when it was normal unless infection developed, when the injection of the fine arterial branches was incomplete. ADAMS, HRDINA and DOSTAL (1935) found this reduced filling even in the absence of infection. FINE and DRINKER (1931) perfunded animals with nickel-sulphide and by a quantitative nickel analysis of the pulmonary tissues they found a reduced blood-flow through the lung during pneumothorax and during atelectasis caused by bronchial occlusion. Injection of colloidal solution of graphite in rabbit with atelectatic areas in the lungs produced scarcely any colouring of these parts (HANSON and SJÖSTRAND 1935). As a general result of these injection experiments, it seems highly probable that the blood-flow through an atelectatic part of the lung is less than the normal.

Better possibilities for a quantitative determination of the blood-flow through collapsed and infiltrated parts of the lung seem to be offered by an investigation technique first introduced by SACKUR (1895, 1897). When mixing fully oxygenated blood with venous blood from non-ventilated parts of the lung the oxygen content of the arterial blood is reduced. If the oxygen content of the blood coming from ventilated alveoli is known as well as the oxygen deficit of venous and mixed arterial blood, it is then possible to calculate the proportion of blood passing non-ventilated parts. In

the event of open pneumothorax, where SACKUR pre-supposes that one lung is completely collapsed and the other ventilating in full extent, the relation between the blood-flow through the two lungs may be calculated according to the following formula:

$$\frac{\text{right lung}}{\text{left lung}} = \frac{\text{pa} - \text{pl}}{\text{pr} - \text{pa}}$$

(pa oxygen content in arterial blood from carotids during pneumothorax, pr oxygen content of blood from the right lung (the functioning lung), pl oxygen content of blood from the left lung (pneumothorax side)). Assuming that the blood from the functioning lung is 100 % saturated with oxygen and that the oxygen deficit of the venous blood is 50 %, SACKUR comes to the result in two cases that the blood-flow through the collapsed lung is greater than that through the functioning one. The principle for this method of determining the blood-flow through non-ventilated parts of the lung seems to be irreproachable, but the results which the author arrives at seem open to question. When the pneumothorax is open the ventilation of the lung on the other side is affected considerably as pointed out by WEISS (1926) and TÖRNING (1933) and others. SACKUR's assumption that the blood from this lung is saturated to 100 % is therefore probably erroneous.

By the same manner of determination, HESS (1912) found that the blood-flow through a lung, the bronchus of which had been obstructed, is about the same as when the lung ventilates freely. LE BLANC (1922) came to the same conclusion in similar experiments. In an extensive investigation of the effect of the open and closed pneumothorax on the oxygen content of the arterial blood of rabbit and dog, WEISS (1926) found that in rabbit a decrease in the oxygen content occurs only in the case of an open pneumothorax, while in dog a decrease takes place when the pneumothorax is closed as well as open. Shutting off the blood supply on the side of the open pneumothorax by ligating the hilum produced in some cases only a moderate increase in the oxygen content of the arterial blood, which indicates a slight blood-flow through the collapsed lung. In other cases, however, the increase was considerable. By means of oxygen inhalation it was possible in most cases of decreased arterial oxygen saturation to regain a normal value, and in consequence WEISS draws the conclusion that in cases of pneumothorax anoxemia is chiefly due to an incomplete oxygenation of the blood in the free lung on account of irregular ventilation

and pendulum air. In attempts to make calculations according to SACKUR's principle in the case of bronchial occlusion in man during broncho-spirometry JACOBÆUS and BRUCE (1940) in some experiments found that the blood flow through the occluded lung was almost unchanged, in others, however, quite unlikely values were obtained.

According to ANDRUS (1925) the blood-flow is somewhat diminished immediately following the occlusion of the bronchus, and afterwards decreases still more with the age of the atelectasis. The calculation was carried out according to FICK's principle from the elimination of carbon dioxide and the difference in carbon dioxide content between venous and arterial blood. A value for the blood-flow thus calculated does not, however, represent. as ANDRUS seems to consider, the circulation through the ventilating lung alone but the entire circulation. In his calculations the author assumed the cardiac output to be the same before and after the bronchial occlusion. Instead of proving something concerning the blood-flow through the atelectatic lung, his determinations show a decreased cardiac output as the result of occlusion, which decrease subsequently disappears.

In a series of experiments on dog in which a tube was placed in each of the bronchi, MOORE (1931) found that with the occlusion of either bronchus, the blood-flow through the occluded lung, calculated from the decrease in the oxygen content in the arterial blood, clearly diminished. While the ratio between the blood-flow through the right and the left lung in a normal state was 61 : 39, after the occlusion of the left bronchus it was 82.5 : 17.5. The deviation from corresponding experiments by HESS (1912) and LE BLANC (1922) was explained by the fact that the author made the animals breathe oxygen, thus making the lung become atelectatic within a few minutes of the occlusion. Only those cases were included in the calculations in which the oxygen content of the arterial blood in the normal state exceeded 100 % saturation according to the mode of calculation by VAN SLYKE, which means that there was a surplus of physically dissolved oxygen. When breathing pure oxygen, in consequence of this physically dissolved oxygen, its content in a normal state should correspond to a saturation of about 107 %. The fact that the values of saturations in MOORE's experiments were in so many cases considerably below this value, even before the bronchial occlusion, render his values less acceptable. In spite of this and of considerable individual

variations, MOORE's investigations should, on account of the number of his experiments, be regarded as the most reliable work carried out on this subject.

TÖRNING (1933) also determined the arterial oxygen saturation before and after the establishment of a pneumothorax, and in experiments on rabbit with an open pneumothorax it was evident that the share of the collapsed lung in the circulation is considerably reduced. Similarly to WEISS (1926), TÖRNING also found that the decrease in the arterial oxygen saturation resultant from an open pneumothorax was only partly suspended if the circulation through the collapsed lung was entirely excluded by shutting off the vessels in the hilum. In a number of experiments with a closed pneumothorax, not only was the oxygen absorption by each lung determined but also the oxygen saturation of the arterial and venous blood, and from these values the share of each lung in the circulation was calculated. In addition to these values there was a fraction of the circulation, characterized by the author as the unknown component, which regularly increased with the pneumothorax. What TÖRNING means by this component is not quite clear, but in all probability it refers to the amount of blood passing non-ventilated areas. In his experiments TÖRNING used pure oxygen in the inspired air, in order, as he expressed it, to discover as much of the circulating blood as possible. By this he evidently means that the oxygen content even in badly ventilated parts will be sufficient to allow of a complete oxygenation of the blood.

TÖRNING (1933) considered that the anoxæmia following on a pneumothorax is not due to an increased blood-flow through the collapsed lung areas, but can be explained by an imperfect oxygen absorption in the ventilating lung; the anoxæmia therefore disappears when oxygen is inhaled. That some investigators (SACKUR 1895, 1897, HESS 1912, LE BLANC 1922, DOCK and HARRISON 1925), when determining the blood-flow through the collapsed lung by analysing the arterial oxygen saturation, have come to results indicating an unchanged or a more prolific blood-flow through the collapsed lung was considered by TÖRNING to be due to their not having taken into consideration this impaired oxygen absorption in the ventilating lung. The arterial oxygen saturation previous to the experiment is therefore an incorrect starting value for the calculation of the blood-flow through non-ventilated lung areas. Instead, this starting value should be put lower, thus giving lower values for the blood-flow in question.

Determination of Admixture of Venous Blood to fully Arterialized Blood.

In order to be able to establish, according to SACKUR's principle, the existence of blood which has not absorbed oxygen during its passage through the lung, it is absolutely necessary to possess an exact knowledge of the oxygen content in the blood that has been oxygenated in an ordinary manner. Even in normal conditions arterial oxygen saturation varies some per cent. A saturation of 94—97 % may be set as a normal sphere of variation (oxygen capacity is then determined by the saturation of the air at room temperature, by means of which a component of physically dissolved oxygen is added to the chemically combined, for which correction is not usually made (see PETERS and VAN SLYKE 1932)), and not until the saturation is below 92 % may it be reasonable to assume that it is a question of a pathological value. It is erroneous to consider that a decrease in the saturation compared with an assumed normal value is an expression of admixture of venous blood, because this decrease may also be due to other causes, as was pointed out in the introductory chapter. In order to obtain a method by means of which it is really possible to isolate the admixture of venous blood as being the only factor that affects the deviation from the normal oxygen saturation, it is necessary to devise experimental conditions in such a manner that the other factors affecting the arterial oxygen content, i. e. diffusion difficulties and insufficient ventilation are as far as possible eliminated.

This object is simply attained by using pure oxygen instead of air. As has already been mentioned, MOORE (1931) and TÖRNING (1933) availed themselves of this possibility, but they have not made full use of the favourable consequence that oxygen breathing ensures for this problem.

By disposing of all nitrogen and replacing it with oxygen, the irregular ventilation as a factor causing anoxæmia disappears. If all nitrogen is eliminated, oxygen cannot sink below a value of 100 % minus the carbon dioxide content, and the latter cannot rise to a higher level than that which corresponds to its tension in the venous blood, which is c. 6 % of an atmosphere. In order to make sure that the greatest part of the nitrogen really has been eliminated from a badly ventilated part, oxygen inhalation must

naturally be going on for a fairly good time before the test is made.

A decrease of the diffusion velocity through the alveolar epithelium will also lack importance within fairly extensive limits. The chemically combined oxygen which constitutes the greater part of the oxygen that has to diffuse into the blood, exerts a tension between 40—100 mm. the difference of tension existing for the diffusion will thus be c. 600 mm. For the diffusion of 200 ml. oxygen per min. the necessary diffusion constant is c. 0.3. In order to bring the oxygen tension of the blood in equilibrium with the alveolar air to within 1 mm. an increase of the diffusion constant by 0.8[1] will be necessary. The resulting value of 1.1 is only 1/20 of the normal minimum value (24 ml. O_2/min. see p. 11). A diffusion constant of this magnitude is incompatible with the existence of life when breathing ordinary air.

This considerable margin is to be found when the diffusion through all parts of the alveolar wall is equally great. Under normal conditions no great deviations from this uniformity are to be expected, but in pathological circumstances the existence of all transitions are conceivable from normally transmittable alveolar epithelium to such epithelium as by thickening and exudation under normal oxygen tension is practically impenetrable for this gas. With the considerable rise of the oxygen tension that oxygen breathing causes, there are possibilities for such alveoli to transmit some oxygen. Thus there is a sphere where diffusion difficulties and the admixture of venous blood meet as a cause of arterial oxygen deficit. The quantitative significance of this sphere of contact must remain unestablished for the present.

The magnitude of the arterial oxygen deficit, which is the result of the admixture of venous blood to the fully oxygenated, will be best understood by an example. With an arterio-venous difference of 60 ml. pr l., the oxygen content of the arterial blood will sink by 0.6 ml. pr l., i. e. 0.06 vol.% with every per cent of the

[1] The diffusion of physically dissolved oxygen can be expressed by the following formula (BOHR 1909, p. 253):

$$\frac{d}{M} = \frac{\alpha}{760} \ln \frac{P - p_v}{P - p_a}$$

(d = diffusion constant. M = blood-flow/min. α = solubility coefficient. P=alveolar oxygen tension. p_v = venous oxygen tension. p_a = oxygen tension of arterialized blood). If M = 4 l. $\alpha = 0.023$ ml. P = 675 mm. p_v = 40 mm. and p_a 674 mm. (i. e. the diffusion proceeds to a final tension difference of but 1 mm.), d is 0.8 ml. O_2/ min.

total blood-flow that is not arterialized. Making a blood-gas analysis in VAN SLYKE's and NEILL's manometric analysis apparatus, it is possible after long training to attain an accuracy of c. 0.1 vol.%. With this method, which nevertheless signifies the most accurate available for such analyses, it is not possible to reach the lesser degrees of this admixture of venous blood.

When breathing pure oxygen the fraction of physically dissolved oxygen amounts to c. 2.0 vol.% while when breathing ordinary air it amounts to only 0.26 vol.% (LILJESTRAND 1928). In order to saturate the hemoglobin of the admixed venous blood with oxygen, this surplus of physically dissolved blood is used in the first place. Only when such a large quantity of the physically dissolved oxygen has been consumed that the oxygen tension sinks to c. 150 mm. can there be a certain incomplete oxygen saturation of the hemoglobin. The surplus of physically dissolved oxygen amounts to 1.6 vol.%. With an oxygen deficit of venous blood of 6 vol.%, this surplus is sufficient for the complete saturation of the hemoglobin of an admixture of venous blood which amounts to 27 % of the total blood-flow of the lung (see fig. 12 p. 53. cp. PETERS and VAN SLYKE I p. 583).

By a determination of only physically dissolved oxygen it would thus be possible to determine the admixture of venous blood to the fully arterialized blood within fairly extensive limits. The chemically combined oxygen is easily separated by centrifuging the blood-cells. According to SPEITKAMP's (1939) investigations of centrifuged blood, it was found, it is true, that in plasma only a small quantity of the physically dissolved oxygen could be discovered. SPEITKAMP himself interpreted his results as a sign of an adsorption of oxygen to the blood-cells in agreement with a theory by CONANT and SCOTT (1926). According to exceedingly accurate investigations of the physically dissolved oxygen which have been carried out by SENDROY, DILLON and VAN SLYKE (1934), however, there are no indications of any adsorption process. The determination of nitrogen in SPEITKAMP's investigation showed very nearly the expected amount. This is in remarkable contrast to the adsorption theory. No signs of the existence of any similar decrease in the oxygen content in plasma after centrifuging have been established in the own investigations.

METHODS.

Methods of Determining Physically Dissolved Oxygen.

As physically dissolved oxygen in blood and other fluids in the body is to be found in very small quantities, the determination involves certain difficulties unless there are considerable quantities of fluids at hand. The manometric apparatus for analysing gas of liquids constructed by VAN SLYKE and NEILL (1924), which is nowadays extensively used, requires for the analysis of physically dissolved oxygen in blood at least 5 ml. plasma for a single determination, which means that the blood sample must be 10—15 ml. When making experiments on man, this is certainly no quantity of blood worth mentioning, but if a series of determinations are made on the usual laboratory animals, the blood samples will be too great. It was therefore considered desirable to work out a method which rendered reliable analyses possible on considerably smaller quantities of blood. In the following pages two processes will be described in detail for these determinations. The one method is a further development of the principle adopted in VAN SLYKE's and NEILL's apparatus, i. e. the extraction of gases in vacuum, and the measurement of pressure at constant volume. The other method is based on the fact that under certain conditions oxygen is reduced at a mercury drop electrode and gives rise to an electric current proportionate to the oxygen content.

Micro-Analysis of Physically Dissolved Oxygen according to the Principle of Van Slyke and Neill.

The basis of the construction of the apparatus about to be described was a method for blood-gas analysis which has been introduced by MOOK (1930, 1931) and which is stated to allow analysis of blood samples down to 50 c. mm. The blood sample is placed in

this apparatus and the liquids necessary for the extraction of gases are put into a separate pipette with a capillary tube closed at one end. The pipette is attached to an evacuation chamber by means of a mercury seal. The extracted gases are measured at atmospheric pressure. The difficulty of getting an uninterrupted pillar of gas in the narrow capillary in MOOK's apparatus is a great disadvantage for the method. This drawback has been eliminated by reconstructing the gas chamber in the form of a bubble and by changing from the measurement of the volume at constant pressure to the measurement of the pressure at constant volume, as has been done with the VAN SLYKE-NEILL apparatus in comparison with VAN SLYKE's previous volumetric apparatus. Another source of error that appeared when attempting to analyse still smaller samples than it was possible to do in MOOK's apparatus was the extraction of gas from the liquid which adheres to the glass joint of the pipette. This liquid is not entirely eliminated when the pipette is put down into the mercury seal. Some gas is also adsorbed by the joint and extracted when this is put under vacuum. These errors, too, have been eliminated by putting gas and evacuation chambers together into the detachable pipette.

The final appearance of the apparatus is seen in fig. 1. The gas chamber with a cubic volume of 25 c.mm. is attached to an evacuation chamber of 4—5 ml. The pipette is attached to a glass tube by means of a 3 cm. long neck with a glass joint. Immediately above this joint the tube widens to narrow off again round the upper part of the neck of the pipette to form a mercury seal, which remains in position during the shaking (fig. 2 C). Above the mercury seal the tube again expands into a container in which there is water to retain the temperature constant. The junction used below the evacuation chamber in VAN SLYKE-NEILL's apparatus (a double rubber tube with mercury in the intervening space) has been replaced by a rubber junction in the shank rising to the ┼-cross. The shank falling from the evacuation pipette will thus consist of an uninterrupted glass tube. Even if it is likely that NEILL's junction is absolutely reliable after having stood under vacuum for some time, this part which is inaccessible to inspection gives a feeling of uncertainty which is entirely avoided with the junction here used. Any air that might possibly penetrate the rubber can never come up into the pipette but accumulates in the upper shank of the ┼-cross, in which air from the rubber tube attached to the mercury levelling bulb is also accumulated. The construction of the hanging and shaking arrangements necessary for this junction is seen in fig. 1. The most important detail here is to see that the axis round which the movement takes place intersects the junction, so that all strain on the glass parts is avoided and no change in the position of the mercury possible. This can be easily done by fixing the shafts round which the turning takes place

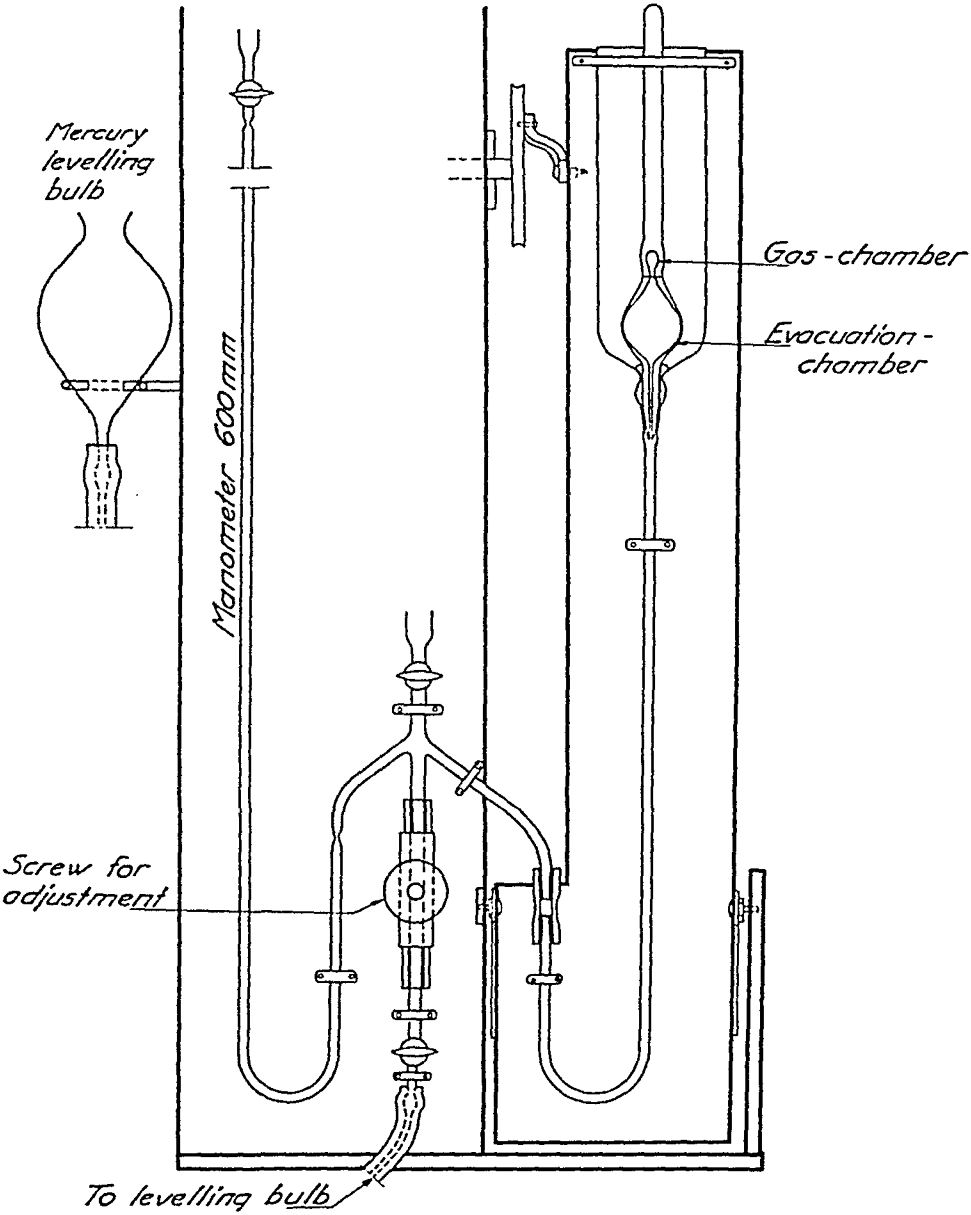


Fig. 1. Apparatus for micro-analysis of blood-gases according to evacuation method.

into an angle-bar, one shank of which projects so far that the shaft will be on a level with the rubber junction. It is also advisable to fit the apparatus with a screw for adjustment placed immediately above the stop-cock to the mercury levelling bulb. The adjustment can also be made by means of an ordinary rubber screw-clip placed at the junction above described.

If the shape of the gas chamber is unsuitable, a liquid meniscus would be likely to stick on the inside and thus completely derange the measurements. The ideal shape is a bubble with a cross-section, the curve of which has a radius increasing successively from the top (see fig. 2 B). It must not, however, be too extended, for the surface should be as small as possible in comparison to the volume. The narrow connection between the gas and evacuation chambers should be as short as possible. In order not to make the glass-blowing work unnecessarily difficult, it is better to insist on the ideal shape and let the volume be approximately 25 c.mm. There is no difficulty in correcting a volume that is not exactly that measurement. The inside diameter of the narrow connection between gas chamber and evacuation chamber should be 1 mm. and of the neck of the pipette c. 1.5 mm. The lower part of the pipette is pointed, thus forming a cul-de-sac round the opening, which gives greater security against outside gas penetrating into the pipette. The gas which is collected in this cul-de-sac comes, as has already been stated, from liquid adhering to the joint and from gas adsorbed by this.

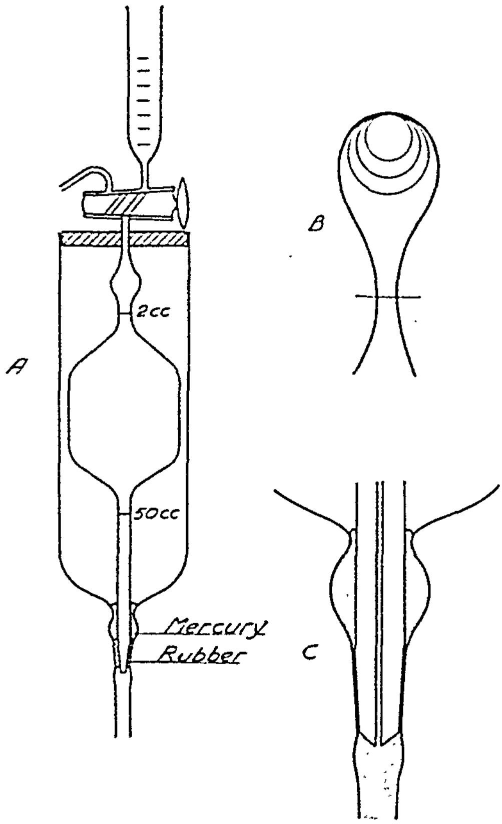


Fig. 2. Details of apparatus.

A. Attachment of pipette for usual Van Slyke analysis.
B. Section through gas chamber.
C. Mercury seal.

This formation of gas can be eliminated by polishing the joint of the pipette and by using evacuated water above the mercury. These precautionary measures are not necessary, however, as these gases cannot penetrate up into the pipette unless it is completely emptied of mercury at the time of evacuation. The handle of the pipette should not be made of solid glass but of a glass tube, so as to avoid unnecessary strain on the joint during the shaking.

Instead of this pipette for micro-determinations an ordinary Van Slyke-Neill evacuation pipette can also be attached (fig. 2 A). The apparatus can then be used for ordinary Van Slyke analyses, and the evacuation of the necessary solutions requires no special apparatus. A glass joint is not necessary for the junction, but a rubber packing

can be used as indicated in this fig. The point of the pipette should go down a little below the rubber packing so as to prevent air from this packing from coming up into the pipette. Before the apparatus is used for analysis, the junction is placed for a little while under vacuum by lowering the mercury below the junction, thus extracting the greater quantity of the gas that is in the rubber packing.

The evacuation of the solutions (alkali, FIESER's solution for oxygen absorption, i. e. sodium hypo-sulfite + sodium antraquinone beta-sulfonate, octyl alcohol) takes place according to the instructions in PETER's and VAN SLYKE's (1932) description of blood-gas analysis. The evacuated solutions are transferred into glass syringes, which are rinsed 2—3 times with the liquid evacuated. Glass capillaries are used as syringe needles. These glass capillaries are fragile, it is true, but they make it possible to control that there are no air bubbles in the liquid when it is injected into the pipette. The long glass capillary renders diffusion of air into the syringe impossible. By grinding a circular groove in the piston of the syringe and filling it with mercury, an absolute protection for the evacuated solution is easily obtained. Almost gas-free water is obtained by boiling a few minutes. The glass syringe is filled while the water is boiling.

An analysis of the oxygen and nitrogen existing in plasma is made in the following manner.

The gas chamber is filled with evacuated water, the evacuation chamber, with mercury. In order to get rid of the gas bubbles sticking to the walls and to replace the water that has come into contact with the air, the pipette is flushed from the gas chamber with the evacuated water. The pipette meanwhile is held neck upwards. While these flushes are going on, it is important to see that no mercury slips into the gas chamber and then sticks like a stopper in the narrow connection. This is most easily avoided if the capillary is inserted while the pipette is held horizontally and is not changed into a vertical position until the flushing begins. If by any chance a drop of mercury should get into the gas chamber, it may be removed by injecting water while keeping the chamber turned upwards. A certain squirting out of mercury from the pipette cannot be avoided, and therefore the manipulations should preferably be done over a bowl of water. A little drop of octyl alcohol is put into the gas-chamber to prevent foaming. 0.2 ml. plasma is placed in the evacuation chamber with the help of a screw-pipette. The pipette must be in a horizontal position, otherwise air will easily get in or the plasma will run out, depending on whether the point of the pipette is directed downwards or upwards. The neck of the pipette is filled with mercury and it is now put down into the mercury seal, the water mantle is filled with water, and for the purpose of getting vacuum the levelling bulb is lowered till the mercury in the manometer stands on a level with the middle of the evacuation chamber. It often takes a couple of minutes for the gas development to get started, but this may be hastened by gently knocking the suspending disc. By resuming atmospheric pres-

sure for a moment the bubble first formed will rise into the gas chamber. When the levelling bulb is lowered, the liquid in the chamber will also sink. The level of the mercury is lowered to the upper opening of the neck of the pipette. Under no circumstances must the mercury sink below the end, for then the air that is in the cul-de-sac round the point of the pipette will always follow the mercury when this rises again. After shaking for three minutes the stop-cock for the levelling bulb is opened, and the extracted gases (carbon dioxide, oxygen and nitrogen) are returned to atmospheric pressure. The pipette is detached and 0.04 ml. 1-N NaOH solution is injected into the evacuation chamber, the pipette being kept horizontal. When the neck of the pipette has been filled with mercury it is again put into the seal, and the mercury is once more lowered to the upper opening of the neck of the pipette. After shaking for one minute, during which time the carbon dioxide has become completely absorbed, the level of the liquid is carefully adjusted to the line below the gas chamber, a possible meniscus formation in this chamber having been removed by letting the gas bubble resume atmospheric pressure for a moment and rise to the top of the gas chamber. When the pipette has again been detached, 0.01 ml. FIESER's solution is injected straight into the gas chamber, and the pipette is rotated in a horizontal position until the gas bubble no longer diminishes in volume, which process only takes about a minute. The neck of the pipette is again filled with mercury and after one minute's evacuation for the extraction of the nitrogen which has been dissolved during the absorption of the oxygen, a second adjustment and reading is made.

The reason why the alkali is not applied previous to the evacuation, the carbon dioxide not being determined in any case, is the strong increase of the oxygen consumption of the plasma which takes place when this becomes alkalified. By using 0.1-N alkali this consumption of oxygen becomes less, it is true, but it is still a great source of error. If alkali is injected straight into the gas chamber when the gases have been extracted by evacuation, this also involves a systematic error, because the oxygen dissolved during the absorption of the carbon dioxide seems for the most part to be consumed and is not recovered by renewed evacuation. With the above mentioned method the extracted gas bubble is sufficiently large to almost entirely fill the gas chamber, thus making the surface of contact between gas and liquid insignificant. The concentration of oxygen in this gas bubble is, moreover, relatively low, as the greater part of the gas extracted being carbon dioxide. By placing the alkali in the evacuation chamber, the plasma does not become alkalified until it is again under vacuum, when there is no longer any possibility for the oxygen to be dissolved. In the accurate determinations of the physically dissolved oxygen carried out by SENDROY, DILLON and VAN SLYKE (1934) the carbon dioxide is likewise not absorbed until the gases have been extracted, but no motive is given for this procedure.

In order to determine the nitrogen percentage, it is necessary to know the position of the manometer when there is no gas in the chamber, and this involves certain difficulties. If the pipette is filled with com-

pletely evacuated liquids, there will never be any gas bubble. A satisfactory conception may be formed by inserting a minute air bubble by means of a glass capillary and then making a reading. The amount of the liquid in the pipette should correspond to that which exists after the completion of an analysis (0.25 ml. beyond the liquid in the gas chamber). If the bubble is sufficiently small (barely visible to the naked eye), the error need not amount to more than some fractions of a millimetre.

In consequence of the small amount of FIESER's solution that is used, it is not necessary to make any correction of the manometer values for the change in the mercury level in the pipette.

The calculation of the amount of gas is made with the help of the formula given by VAN SLYKE and STADIE.

$$V_{0^\circ 760} = P \frac{a \cdot i}{760\ (1 + 0.00384\ t)} \left(1 + \frac{\alpha \cdot S}{A - S}\right)$$

P. the difference in pressure between the readings.
a. the volume of the gas chamber.
i. the reabsorption factor (which for oxygen and nitrogen is 1, i. e. no reabsorption).
t. the temperature.
S. the amount of liquid in the pipette.
A. the volume of the pipette.
α. the coefficient of solubility of the gas.

The factor $\frac{\alpha \cdot S}{A - S}$ is so trifling for oxygen and nitrogen on account of the low value of the coefficient of solubility that it can be ignored.

The figure 0.00384 is an empiric constant given by VAN SLYKE which includes the effect of temperature on the volume of gas, mercury and glass.

The apparatus can also be used for analysis of the gases in blood. The amount necessary for analysis is 10 c.mm. Further, it is also applicable for the micro-determination of gases down to samples of a few c.mm. The technical details of these analyses will be published later.

Polarographic Oxygen Determination in Blood.

The Polarograph.

The polarograph as a qualitative and quantitative chemical method has been worked out by HEYROVSKÝ and his school. The method is based on the principle that ions existing in a solution are able to be reduced at a mercury drop electrode, provided that a suitable potential is applied. What distinguishes this electrode

from the ordinary stationary ones is that, firstly the electrode surface is being constantly renewed, secondly its surface undergoes a regular change in size with every drop of mercury that is formed, and lastly the part of the solution that comes into contact with the electrode is renewed with every fresh drop formed.

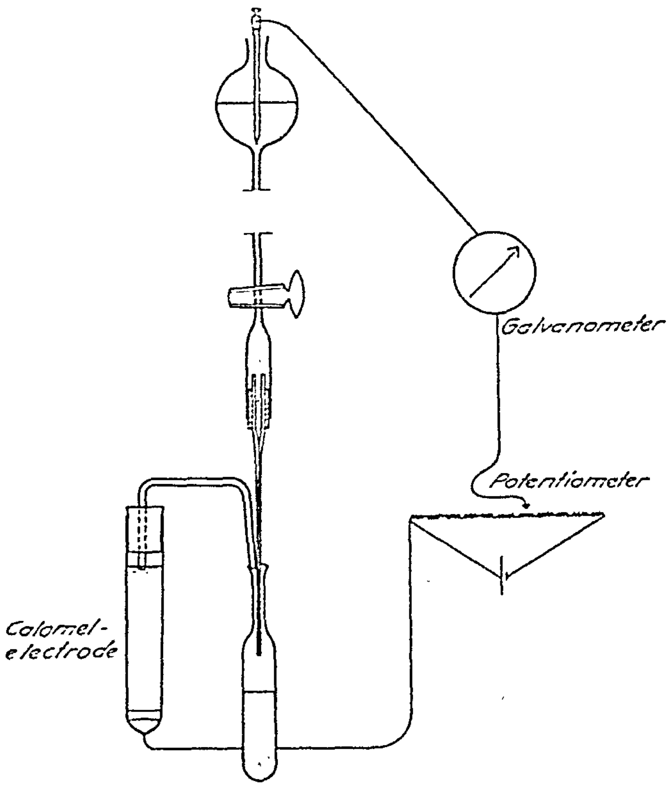


Fig. 3. Arrangement for polarographic oxygen determination in blood.

The current registered on a galvanometer in the outer circle is due to a change of electrons at the electrode and is dependent on the potential and quantity of substances capable of reaction. Apart from positive ions some undissociated substances may also be reduced i. e. take up electrons, and oxygen is one of these.

Besides the capillary which is drawn out to a fine point and through which the mercury flows, the polarograph consists of a mercury container, a galvanometer and a potentiometer and possibly some sort of an automatic registration arrangement (*fig.* 3).

If for a solution containing some different substances a curve is made of the variations of the current when the voltage is successively increased, a characteristic ladder-like curve is obtained. The

potential corresponding to the rapid increase of current is characteristic of each particular substance and depends on the facility with which the exchange of electrons is brought about. The level formation appearing after the period of rapid increase in the current is an expression of a pure diffusion of the reducible particles up to the surface of the mercury drop. When the potential reaches such a value that all the particles coming to the surface of the electrode are immediately reduced, the afflux of particles will depend on the concentration and the diffusion velocity. The condition for the appearance of this so-called diffusion current (ILCOVIČ 1934) is, in the case of electrolytically dissociated particles, that the fall of the potential in the solution is diminished by the existence of an electrolyte, the positive ion of which is not reduced until a higher potential is applied. If such is not the case, a component is added to the diffusion current, a so-called migration current, which is due to the reduction of particles having been attracted to the elctrode, thanks to the fall of potential within the solution. For undissociated molecules, e. g. oxygen, the migration current is excluded entirely (ILCOVIČ 1934).

A tabulation of the potential characteristic for the reduction of different substances gives a so-called polarographic spectrum, which is analogous to standard electrode potentials. The oxygen is reduced already at a low minus potential (— 0.4 V. in neutral solution, while for example sodium is not reduced until — 1.8 V.). Within the same area of potential where the oxygen is reduced, Fe, Cu, Pb and Bi are also reduced. The possible concentrations of these ions in blood are very small and are of no importance in comparison with oxygen. Thus there is a fairly large field of potential, within which the current is caused by the reduction of oxygen only. The applicability of the method for quantitative determination of oxygen in biological media is based on this fact.

HEYROVSKÝ and ŠIMŮNEK (1929) found that the current for the reduction of oxygen in four solutions, in equilibrium with 0, 2.83, 21 and 93.7 %, oxygen stands as 0 : 3.5 : 21 : 91. VÍTEK (1935) has subsequently made systematic use of this proportionality between current and the tension of oxygen for measuring oxygen content in gases. By saturating water or alcohol with the gas mixture to be analysed with subsequent polarographic analysis of the liquid, an expression was obtained for the oxygen tension. Other gases, such as carbon dioxide, hydrogen and nitrogen did not affect the determination. In order to be able to follow uninter-

ruptedly the decrease in the oxygen content during inter alia the metabolism of bacteria, PETERING and DANIELS (1938) and WINZLER (1939) availed themselves of polarographic oxygen determination. For analysing oxygen in the blood, the polarograph seems to have been used only by BAUMBERGER (1940). By converting hemoglobin in hematin the oxygen is released and it is possible to establish the oxygen content in the solution. The quantity of blood necessary is 20 c.mm. and the accuracy of a determination is stated to correspond to the ordinary VAN SLYKE analyses.

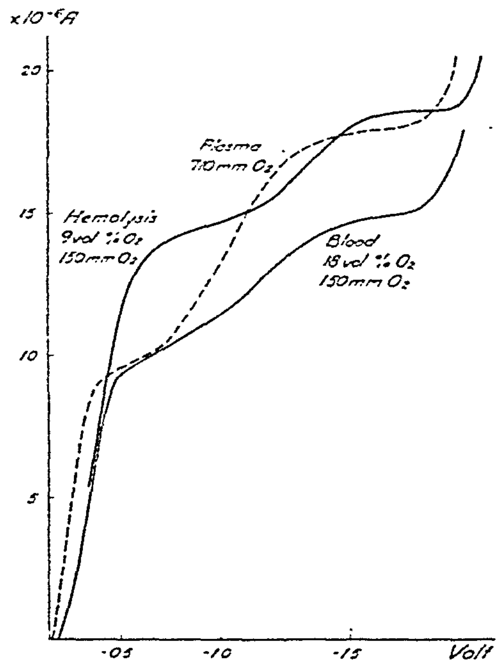


Fig. 4. Comparison between polarographically registered current of oxygen reduction in blood and plasma.

The reduction of the oxygen takes place in two stages, which according to VÍTEK (1935) are represented by the reactions

$O_2 + 2\,e + 2\,H^+ \rightarrow H_2O_2$

$H_2O_2 + 2\,e + 2\,H^+ \rightarrow 2\,H_2O$ where e represents an electron.

The agreement between the second step in the oxygen reduction curve and that obtained on analysing a solution of hydrogen peroxide seems to justify the assumption that hydrogen peroxide appears as an intermediate stage. The typical course of the oxygen reduction is illustrated in fig. 4.

Polarographic Oxygen Determinations in the Presence of Chemically Combined Oxygen.

In attempts to apply the polarograph for the study of oxygen in the blood, particularly the physically dissolved oxygen in plasma, it has been considered suitable also to examine more closely what takes place with blood under different conditions. As the diffusion current is an expression for the diffusion velocity, which depends on the oxygen tension, it was at first thought theoretically possible to directly obtain an expression for the oxygen

tension in blood, but it was soon found that the oxygen which was chemically combined with the hemoglobin is partially released and increases the current of the oxygen reduction.

According to BRDICKA and TROPP (1937), hemoglobin catalyses the reduction of hydrogen peroxide, so that the reduction begins at a lower minus potential than it does normally. With a sufficiently high percentage of hemoglobin the steps of reduction of oxygen and hydrogen peroxide merge into each other. The total height of the two reactions is not, however, affected. The catalysis is most evident at lower *p*H.

Under certain conditions curves of the same appearance have been obtained as these authors described, e. g. when mixing hemoglobin crystals with a phosphate buffer solution, or in hemolyzed blood, in which the hemoglobin has been blocked by CO. In blood in which oxygen occurs in both chemically and physically combined form, whether the blood is hemolysed or not, a two-step curve will always appear. In contrast to the curve obtained in plasma free of hemolysis, the first step is, however, raised in comparison to the second, as is seen in the curves in fig. 4. That it concerns a rising of the first step is evident from a comparison between plasma free of hemolysis and plasma in which an hemolysis has taken place (table VI page 48). A statistically highly significant rise of the first step is to be found here, while the second one lies within the normal sphere of variation.

Variations of the amount of chemically combined oxygen at a constant amount of physically dissolved oxygen causes corresponding variations in the polarographically registered current, but the proportionality is not rectilinear (see fig. 5). In this series as in the ones immediately following, the reading has been made on the second step at a potential of — 1.6 V. as compared with a saturated calomel electrode. From the curve it will be seen that the increase of the current, which is to be ascribed to the chemically combined oxygen, by no means corresponds to the increase in oxygen content. While at normal oxygen capacity the blood contains c. 40 times as much chemically as physically combined oxygen, the current in blood is only c. 4 times greater than it is in plasma.

If instead the degree of saturation is varied by bringing blood in equilibrium with different oxygen tensions, a more rectilinear course of the curve is obtained within the sphere for incomplete saturation (fig. 6). When the tension is exceeded at which the hemoglobin is completely saturated with oxygen, it is only the

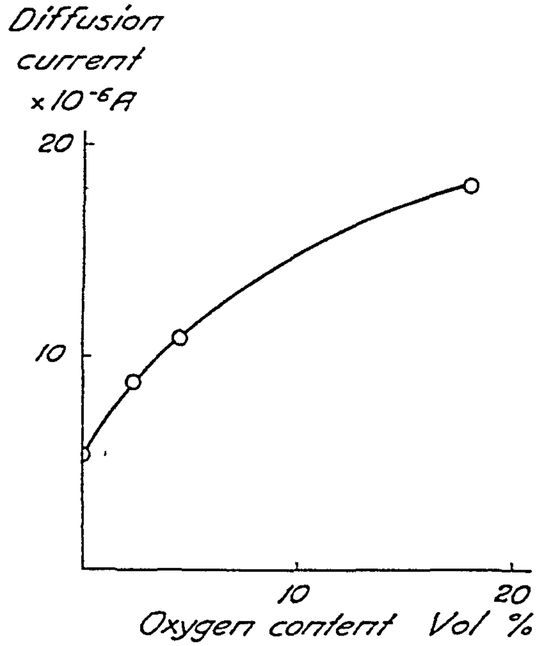


Fig. 5. Diffusion current of oxygen at constant oxygen tension (150 mm.) but varying quantities of chemically combined oxygen.

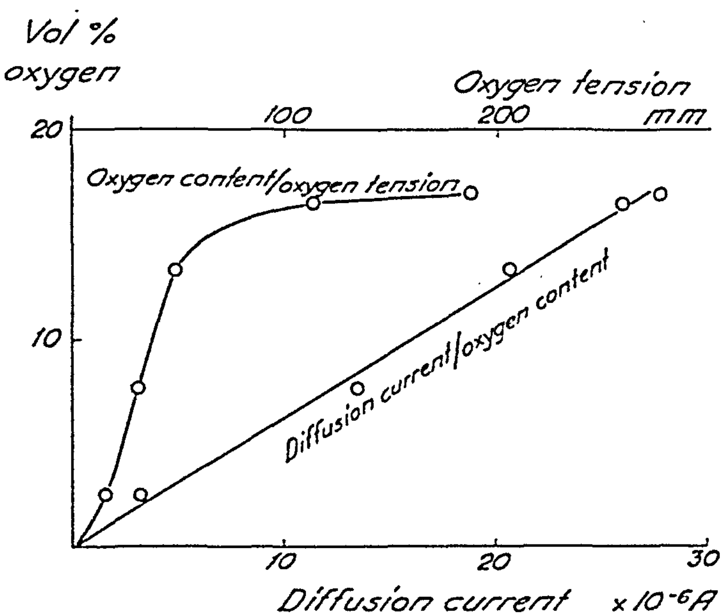


Fig. 6. Diffusion current of oxygen and oxygen content in blood at varying oxygen tensions.

physically dissolved oxygen that increases. At these higher tensions the polarographically registered current follows the physically dissolved oxygen.

The effect of the variations in temperature on the current produced by oxygen will be seen in fig. 7. As is to be expected from the kinetic gas theory, the current diminishes with falling tem-

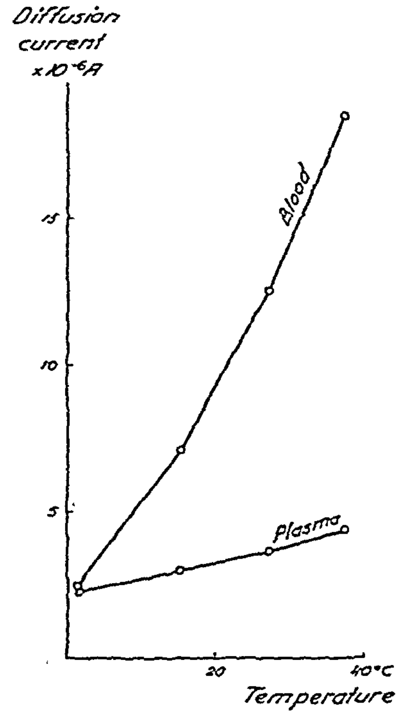


Fig. 7. Diffusion current of oxygen in blood and plasma at varying temperatures.

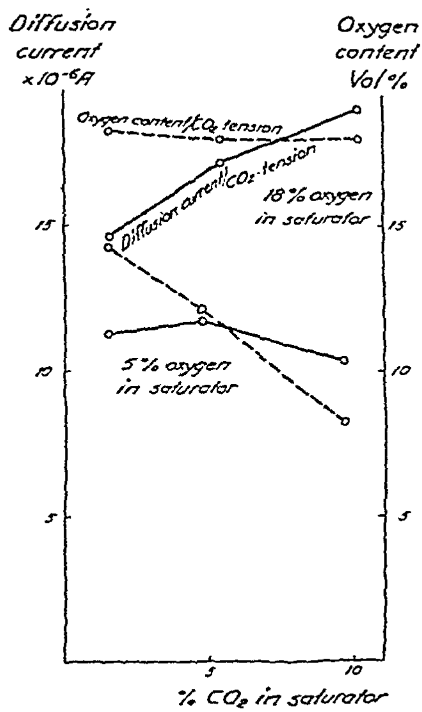


Fig. 8. Influence of variations in carbon dioxide tension on diffusion current of oxygen in blood.

perature. Compared with plasma, where there is only physically dissolved oxygen, this decrease in current is considerably stronger in blood or hemoglobin solutions. At 0° C. the component which is due to the chemically combined oxygen is very small.

While variations in the carbon dioxide content do not affect the current produced by the physically dissolved oxygen (HEYROVSKÝ 1936, VITEK 1935) and are consequently of no importance when measuring on plasma, an increase of the carbon dioxide tension in blood at unchanged oxygen tension brings about an obvious increase of current, as will be seen in fig. 8. Within the sphere of incomplete saturation the diminished affinity between hemo-

globin and oxygen caused by increased carbon dioxide tension must be taken into account, but both experiments show how the curves for current and oxygen content cross each other.

Table I.

The influence of blood-cells on diffusion current of oxygen.

The hemoglobin is blocked by carbon monoxide.
Gas in saturator 45.2 % O_2 = 342 mm.
52.5 % Co
Diffusion currents at −1.6 V (relative values).

Blood	Plasma	Cells
115	130	97
120	125	99
118	129	99

In table I a comparison has been made of some experiments in which the oxygen capacity of hemoglobin has been eliminated by saturating the samples in a mixture of carbon monoxide and oxygen. The oxygen in these samples is thus only physically combined. According to investigations made by SENDROY, DILLON and VAN SLYKE (1934), the solubility coefficient of oxygen is c. 1.20 times greater in hemoglobin than in water. Thus in spite of the fact that the sample with blood-cells contains more physically dissolved oxygen than the other samples, the current is lower. The dilution of plasma with physiological saline causes an increase of the current (table II).

According to the theory for polarography, the current, when the horizontal level has been reached, is an expression for the diffusion velocity of the reducible substance in the solution in question. The diffusion velocity, however, is not only a function of the concentration gradient, but is also due to the obstacles which the solution and the therein existing substances represent to the diffusing particles. This will probably explain the experiments last mentioned, and would thus seem to be a result of the diffusion being slower in the blood-cells and through the blood cell-membrane than is the case in plasma, and besides this, the dilution of plasma colloids facilitates the diffusion.

What takes place in experiments in which oxygen occurs in both chemically and physically combined state is perhaps most easily explained if an idea can be formed of what takes place in the

Table II.

Effect of diluting plasma with 0.9 % NaCl solution on diffusion current of oxygen.

Oxygen pressure in saturator 750 mm.

Relative content of plasma	Diffusion current at − 1.6 V. (relative values)	Oxygen content (ml. O_2/ml. sample)
1	51.8	0.0215
1	52.7	0.0212
0.6	54.5	0.0209 0.0213
0.4	54.8	0.0220 0.0212
0.2	55.6	0.0218
0.1	56.7	0.0211

immediate vicinity of the electrode. The reduction of oxygen makes the concentration of this gas at the electrode surface equal to 0. With the presence of only physically dissolved oxygen, molecules from the surroundings diffund towards the electrode surface and partially fill the vacuum of oxygen caused by the reduction. If, however, there is also chemically combined oxygen present, some of this will be given off within the sphere of decreased oxygen content in order to restore the balance between chemically and physically combined oxygen. The rapidity with which the oxygen is emitted is due to the dissociation velocity of the oxy-hemoglobin and to the diffusion in blood-cells and through the blood-cell membrane. If a comparison based on these observations is made between the above mentioned experiments and the skilful investigations in the kinetic of the hemoglobin carried out by Hartridge and Roughton (1923—1927) they are easy to explain. By quickly mixing oxy-hemoglobin and a reducing solution (sodium hypo-sulfite) and then letting it pass through a long glass tube, these authors, by spectroscopically following the relation between hemoglobin and oxy-hemoglobin at different distances from the mixing chamber, could form an idea of the dissociation velocity for oxy-hemoglobin. This dissociation takes place in the form of a mono-molecular reaction, i. e. in direct proportion to the quantity of oxy-hemoglobin. The rectilinear course of the current in relation to the oxygen content in fig. 6 p. 32 coincides with this.

The decrease of pH causes an increase of the dissociation velocity of oxy-hemoglobin. The larger current when increasing the carbon dioxide tension at constant oxygen tension may probably be explained by a similar pH-effect (fig. 8). The comparison between the velocity with which oxy-hemoglobin is formed from oxygen and hemoglobin, partly by using a hemoglobin solution and partly by blood-cell suspension showed that the reaction in the last mentioned case takes place with only 1/10 of the velocity in the hemoglobin solution. In a theoretical analysis of the co-operation between diffusion velocity and chemical reaction velocity, ROUGHTON (1932) calculated that the reaction velocity for the dissociation of oxy-hemoglobin when the hemoglobin occurs in the blood-cells, is reduced to a good 50 % of the reaction velocity in a hemoglobin solution. The polarographically registered current being almost as great in a sample with intact blood-cells as in hemolyzed blood containing only half of the quantity of hemoglobin, is in fairly good agreement quantitatively with this value (see fig. 4). The effect of the temperature variations on the current (fig. 7) is likewise in qualitative agreement with the experiments of HARTRIDGE and ROUGHTON.

Quantitative Oxygen Determinations by Means of the Polarograph.

In order to be able to obtain from day to day reproducible values with quantitative polarographic analysis, it is necessary to work with constant drop times and a constant flow of mercury. According to ILCOVIČ (1934, 1938) the diffusion current is represented by the formula:

$$i = 0.63 \cdot v \cdot F \cdot C \cdot D^{1/2}\, m^{2/3} \cdot t^{1/6}$$

v = velocity, F = Faraday, C = concentration, D = diffusion coefficient, m = flow of mercury per sec., t = drop time.

The formula shows that the diffusion current increases in proportion to the flow of mercury and the drop time. This formula concerns conditions when using a galvanometer with a long period of swing with which the polarograph is usually equipped, and by which the variations in current during the formation of the mercury drop are equalized. When, as in the present experiment, a galvanometer is used with a short period of swing (Multiflex galvanometer according to LANGE; adjusting time 1—2 sec.), the reading is made immediately before the drop falls, when the current

has reached its maximum. Even in these circumstances the relation between the current on the one hand and the drop time and the flow of mercury on the other is valid. Apart from this being established empirically, it will also be clear from the following argument. The current must obviously be proportionate to the surface of the electrode, and as this increases with increasing drop time, provided that the flow of the mercury is constant, the current must increase with increased drop time. Furthermore the

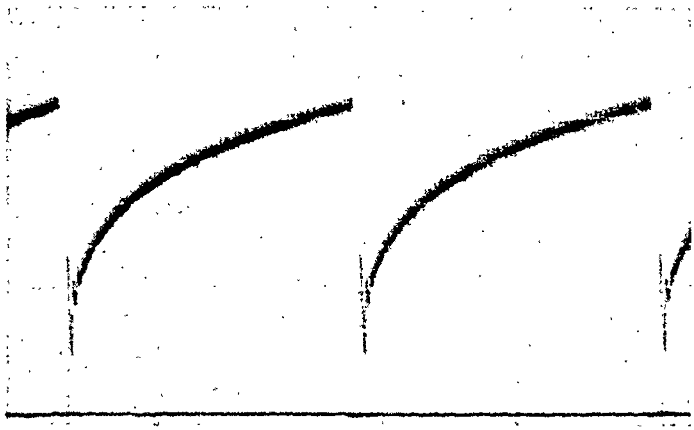


Fig. 9. Variations of current during formation of mercury drop.

current consists of two components, one of which must be attributed to the diffusion of the reducible particles up to the surface of the electrode, the other being due to the growth of the mercury drop, by which the surface of the electrode so to speak meets the diffunding particles (Ilkovič 1934). The greater the flow of mercury, the more rapid will be the increase of the mercury drop and this will consequently produce an increase of the current.

An absolute constancy of the drop time is often difficult to keep, probably on account of minute particles of dirt collecting at the opening of the capillary and affecting the size of the drop. By cleaning the point with nitric acid (25 %) and possibly dipping it into dichromate-sulphuric acid before each analysis a relatively good constancy can be obtained, but in spite of this it is difficult to avoid some slight variations. It is, however, possible to make a correction, because just before the drop falls, the current very nearly follows the time rectilinearly. This will be seen from fig. 9, which is a photographic registration of the variation of the current during the formation of the drop.

Correction for the flow of mercury should naturally also be possible. Repeated determinations of the flow of mercury during a number of days show, however, that this is practically speaking constant, provided of course that the level of the mercury container is not altered. In order to be able to control that variations arrived at in the determinations of the oxygen content are not due to a partial stoppage in the capillary, involving a consequent decrease in the flow of mercury, daily registrations have been made of the flow of mercury during the latter period of these experiments. These registrations of the drop time and the flow of mercury render it unnecessary to make a determination of a solution of standard oxygen content for each analysis, which would otherwise be necessary in order to be able to make a comparison of the values from day to day.

As there should be no temptation to correct the variations in the drop time by altering the level of the mercury container, it has seemed expedient to join it to the capillary, as will be seen from fig. 3 p. 28. The rubber tubing generally used, and which, moreover, represents a great risk for dirting the mercury, has been replaced by a glass tube. By the connection seen in fig. 3 between capillary and glass tube, the former can be easily detached, which is a great asset when cleaning or changing. The direct fusion, advised by KOLTHOFF and LINGANE (1939) would greatly increase the difficulty of cleaning.

Other details with regard to the arrangements for the experiment will also be seen from fig. 3. The determination is made with the sample in a little tube of 1 ml. volume provided with a 15 mm. long neck, the inside diameter of which is fitted to the size of the capillary, so that when this is put down into the tube, there is a sufficient layer of the liquid between the capillary and the wall of the tube to let the electric current pass the liquid without meeting with too great resistance. With a capillary of a good 1 mm. thickness the diameter of the neck has been made 1.7 mm. With all the tubes of the same size the conductive power will be the same in all the samples of a liquid of such an even composition as blood. The conductive power of the solution is in any case sufficient and cannot be decisive for the strength of the current, which is only dependent on the presence of reducible particles, in this case oxygen, at the electrode. The liquid in the test tube is connected to a saturated calomel cell by means of a KCl agar bridge as seen in fig. 3. In order to obtain the different potentials an ordinary potentiometer is used with fixed steps, at every tenth part of a volt. A Lange Multiflex galvanometer is used with an adjusting time of 1—2 sec., which is shunted with a resistance in order to attain a suitable sensitivity. (1 unit = $1.315 \cdot 10^{-7}$ amp.)

In order to protect the samples from coming into contact with air,

which would produce an increase or decrease of the gas content, it has not been possible to use paraffin, partly on account of its tendency to cut off the current, and partly on account of its great solubility coefficient for gases, thus rendering possible a considerable exchange of gas between liquid and paraffin (LOONEY and CHILDS 1934). This is of no importance when ordinary blood samples are concerned, but when measuring small quantities of physically dissolved oxygen, paraffin is a great source of error. In the above test tube every possible exchange of gas with outside air has been avoided by means of the narrow neck through which no diffusion can take place within reasonable time.

If the capillary has been occluded it can usually be made penetrable again by blowing gas from a gas-bomb and sucking dichromate-sulfuric acid and nitric acid through it. After rinsing with distilled water the capillary must be thoroughly dried by warming it over a flame while air is being sucked through. If the end of the capillary gets broken or if a new capillary is needed, calibration with solutions of known oxygen tension will be necessary.

Physically Dissolved Oxygen as an Expression for the Oxygen Tension of the Blood.

As has already been said, the simultaneous presence of both chemically and physically combined oxygen gives rise to such complicated conditions in polarographic measurements that it seems impossible to arrive at a quantitative determination of either the oxygen content or tension directly on blood. The physically dissolved oxygen can, however, be determined by centrifugalizing the blood and making the measurement in the plasma.

The quantity of physically dissolved oxygen in the blood is trifling (c. 1.3 % of the total amount in arterial blood) and has in consequence often been ignored as compared with the considerable quantity of chemically combined oxygen. It should, however, be remembered that the oxygen passes into the tissues in physically dissolved form from the depot in the blood-cells, and that in the lung it is first dissolved physically before it combines with the hemoglobin. In the balance between oxygen tension and oxygen content which is given by the oxyhemoglobin dissociation curve, the oxygen tension is represented by the physically dissolved oxygen, which, according to HENRY's law, occurs in a quantity directly proportionate to the oxygen tension in the gas phase with which the blood is in equilibrium. A quantitative determination of the physically dissolved oxygen thus implies a possibility of coming to a direct expression for the oxygen tension of the blood.

For the direct determination of the tension a method has been adopted which aims at letting a small bubble come into tension equilibrium with the blood, after which the oxygen content of the gas bubble is measured (KROGH 1908, BARCROFT and NAGAHASHI 1921, POULTON, SPURELL and WARNER 1926, MEYER 1935, HICK 1936). The technique is circumstantial, however, and the method has therefore been made little use of. Instead, the tension has been generally calculated from the oxyhemoglobin dissociation curve. If the calculations are carried out by means of the dissociation curve of the blood in question, they will be fairly reliable, particularly in the sphere under 90 % saturation. Above 90 %, however, the rise in oxygen tension is so great as compared with the increase in saturation that slight errors in its determination cause relatively great errors in the calculation (cp. VAN SLYKE-PETERS 1932).

In determining the oxygen tension of the blood by measuring the quantity of physically dissolved oxygen in plasma, the increased affinity between oxygen and hemoglobin at lowered temperature must be taken into consideration at the centrifuging of the blood. If the temperature is below that of the body, a different position of the equilibrium will appear, in which the physically dissolved oxygen will have partly changed into the chemically combined form. In attempts to centrifugalize at the temperature of the body a very unwelcome source of error made its appearance, however, in the form of an oxygen consumption of the blood, and this was found to exist not only in the presence of blood-cells but was quite measurable in plasma free from cells.

It has been known for a long time that a slight consumption of oxygen normally takes place in blood that is kept at the temperature of the body for some hours (MORAWITZ 1909, 1910, DOUGLAS 1909—1910), and also that this consumption increases under certain pathological conditions, such as the increased formation of new blood (MORAWITZ 1909, HARROP 1919, ROESSINGH 1922, DENECKE 1923, TIPTON 1933). That a corresponding consumption also takes place in plasma seems to have been established only under certain conditions, e. g. after treating plasma with ultra-violet rays (HARRIS 1926) or by adding ferricyanide and alkali (PARSONS and PARSONS 1927, LITARCZEK 1928). When making polarographic determinations this consumption of oxygen makes itself noticeable by the decrease in the deflection of the galvanometer, if determinations are made at intervals. At an oxygen ten-

sion of 150 mm. this decrease corresponds to a consumption of c. 0.002 ml. oxygen per ml. plasma per hour, which means a fall in the tension of a good mm. per minute. As warming and centrifugalizing together require c. 7—10 min., an error thus arises which necessitates an extrapolation, and this is a great drawback. By keeping exact times for these procedures, however, it is possible in this manner to form a certain conception or the oxygen tension. Experiments in this direction are not as yet complete, and a closer description is not within the frame of this work.

Attempts to reduce this consumption of oxygen have hitherto only been rendered successful by reducing the temperature of the blood. As has already been said, however, the centrifugalization at low temperature makes a considerable quantity of the physically dissolved oxygen disappear, unless the hemoglobin is fully saturated with oxygen. In normal cases when inhaling ordinary air, there is never such a complete saturation, but in the experimental conditions created when inhaling pure oxygen, as is the case with the method above described for the determination of the blood-flow through non-ventilated parts of the lungs, the hemoglobin is fully oxygenated. Centrifugalization and polarographic reading can then take place at 0° C., and consumption of oxygen is thus entirely eliminated as a source of error.

Comparison between the Polarographic and the Evacuation Method for Oxygen Determination.

When the hemoglobin is entirely saturated with oxygen, i. e. when the oxygen tension is above 150 mm., an analysis of the physically dissolved oxygen in blood is made in the following manner.

The blood is injected into one of the test tubes already described and which has previously been filled with mercury, all contact with air being thus excluded. The mercury which runs out when the tube is filled with blood, should be collected in a basin of water so as to prevent it from dropping round about. The test tube is then transferred into a centrifuging tube, filled with ice water to a suitable height and centrifuged for some minutes. It is then attached to the polarograph and put down into a DEWAR vessel containing ice water. After two or three minutes when it is certain that the temperature has become constant, a reading is made of the galvanometer at the potentials where the levels above described are to be found, i. e. between —0.5 to —1.0 V. and

— 1.5 to — 1.8 V. A determination of the drop time is made in connection with the galvanometer reading at — 0.7 and — 1.6 V. When the polarographic measurement has been completed, an analysis is carried out according to the evacuation method on the same sample. The screw pipette for the evacuation analyses has been filled with the tip as near as possible to the line of demarcation between plasma and the blood-cells, thus eliminating as a source of error in so far as possible the exchange of gases with air. As has previously been mentioned, paraffin is only an illusory protection against a similar exchange of gases and would, moreover, render cleaning more difficult.

In table III a number of determinations are compared in which blood from various normal subjects has been saturated in a BARCROFT's saturator at a temperature of 37° C. with a known gas mixture (92.65 % O_2, 4.45 % CO_2). The excess of pressure arising in the saturator owing to the warming of the air has not been let out, but has been used to inject the sample into the test tube.

The error of the polarographic method is the same whether the reading takes place at — 0.7 V. or at — 1.6 V. and amounts for the single determination to 1 % of the value. HEYROVSKÝ (1936) records the error in the quantitative polarographic analyses to be 5 %, and in the case of oxygen determinations VÍTEK (1934) maintains 2 %. The greater accuracy obtained in this investigation may perhaps be attributed to the fact that the demand for a constancy of the flow of the mercury and the drop time has been more accurately observed. Possibly too the choice of the galvanometer is of importance. With a long period of swing the variations of the current during the formation of the drop are summarized, thus giving the mean current. Occasional variations of the drop time are thereby concealed and make corrections impossible. When using a galvanometer of a short period of swing, as has been the case in this investigation, the maximum current is registered which is reached immediately before the drop falls, and a reading of the drop time is simultaneously taken.

The error of the evacuation method is somewhat greater, but nevertheless it is only 2 % of the value registered. Converted into volumes and the size of the sample duly taken into consideration, the error for a single determination will not exceed 0.08 c.mm. When the nitrogen content of the samples is calculated, a slight systematic source of error appears. The theoretical value for the nitrogen content is 0.00056 ml. pr ml. plasma, and thus there is a mean excess of 0.00022. This surplus of nitrogen will

Table III.

Calibration of normal blood.

Date		Saturator mm. oxygen tension	Polarograph[1]				Evacuation method		
			Galvanometer deflection		mm. oxygen tension per galv. unit.		ml. oxygen per ml. plasma	Solubility coefficient	ml. nitrogen per ml. plasma
			−0.7 V.	−1.6 V.	−0.7 V.	−1.6 V.			
13.2	S. B.	725	26.8		27.1				
		725	27.0		26.9				
		725	27.0		26.9				
		778	28.2		27.6				
		755	28.0		27.0				
		761	28.5		26.7				
14.2		728	27.2	49.3	26.8	14.8			
		731	27.0	49.0	27.1	14.9			
17.2		756	28.2	50.7	26.8	14.9			
		756	27.8		27.2				
19.2		751	28.2	51.8	26.6	14.5	0.0215	0.0218	0.0009
		751	28.5	52.5	26.4	14.3	0.0212	0.0215	0.0010
19.2	J. P.	745	27.7	49.9	26.9	14.9	0.0207	0.0211	0.0010
		743	28.2		26.4		0.0212	0.0217	0.0006
20.2	A.A.	750	28.5	50.9	26.4	14.7	0.0210	0.0213	0.0008
		743	27.8	51.0	26.8	14.6	0.0208	0.0213	0.0006
20.2	L.M.	748	28.2	50.9	26.6	14.7	0.0208	0.0212	0.0006
		753	28.3	50.7	26.6	14.9	0.0210	0.0212	0.0010
20.2	L.G.	735		50.1		14.7	0.0208	0.0215	0.0009
		755		51.6		14.6	0.0213	0.0214	0.0004
20.2	S. B.	748	28.2	51.0	26.6	14.7	0.0208	0.0212	0.0007
		743	28.0	50.7	26.5	14.7	0.0208	0.0213	0.0007
20.2	B.S.	740	27.9	50.2	26.5	14.7	0.0198	0.0204	0.0007
		750	28.0	50.7	26.8	14.8	0.0214	0.0217	0.0007
							0.0211	0.0214	0.0005
21.2	B.G.	756	28.5	51.2	26.5	14.8	0.0220	0.0221	0.0005
		740	28.1	50.9	26.4	14.5	0.0215	0.0220	0.0008
		743	28.3	50.5	26.3	14.7	0.0210	0.0215	0.0006
Mean value					26.70	14.70		0.02142	0.00078
Standard deviation					0.25	0.16		0.00038	
Standard error of the mean . . .					0.05	0.04		0.00009	

[1] One unit on the scale of the galvanometer = 1,315 × 10^{-7} amp. The drop time at − 0.7 V. = 5.0 sec., at − 1.6 V. = 3.9 sec. The flow of mercury = 1.40 mg per sec.

appear to be the result of an admixture of nitrogen during the transmission of the sample to the pipette and the remains of nitrogen in the evacuated solutions. As the error is small and fairly constant, no attempt to correct it has been made.

In fig. 10 there is a comparison of a number of determinations at different oxygen tensions showing the linear connection between the oxygen tension in the saturator on the one hand, the oxygen tension of the plasma determined polarographically and by the evacuation method on the other. When converting the last mentioned values from the galvanometer deflection and oxygen content respectively, the average values from the previous series have been used. As will be seen from table IV there is a difference between the regression coefficients for the values obtained with the polarograph and the evacuation method. This difference is small but can with a probability of only 0.05 be due to mere chance. Comparing the deviations of the separate values from those expected, which are represented by the oxygen tension of the gas phase, no significant difference, however, will appear in either of the methods. The deviation of the separate values in this series is somewhat greater than in the former. The reason for this is partly due to the fact that the same gas mixture has not been used, gas analyses having to be made for each particular sample. The error from the gas analysis, which may be calculated to be c. 0.3 %, will increase the total error.

The linear connection between the galvanometer deflection on the one hand and the oxygen tension in the saturator and the oxygen content in the plasma on the other, undoubtedly prove that in the polarographic determination there really is a specific method for oxygen. That this specificity is no mere chance in the present test, has been established in a considerable number of series, in which the linear connection between current and oxygen tension has been regularly obtained. With complete lack of oxygen there is practically speaking no deflection of the galvanometer until the potential is reached where sodium ions begin to be reduced.

This last series has been carried out on plasma, the separation of the blood-cells having thus been completed before the saturation. When converting the values of current and oxygen content to tension values, the averages have been used that were obtained from the calibration series of blood from normal subjects. Should the centrifuging cause some change in the oxygen content of the plasma, as found by SPEITKAMP (1939), this should appear as a

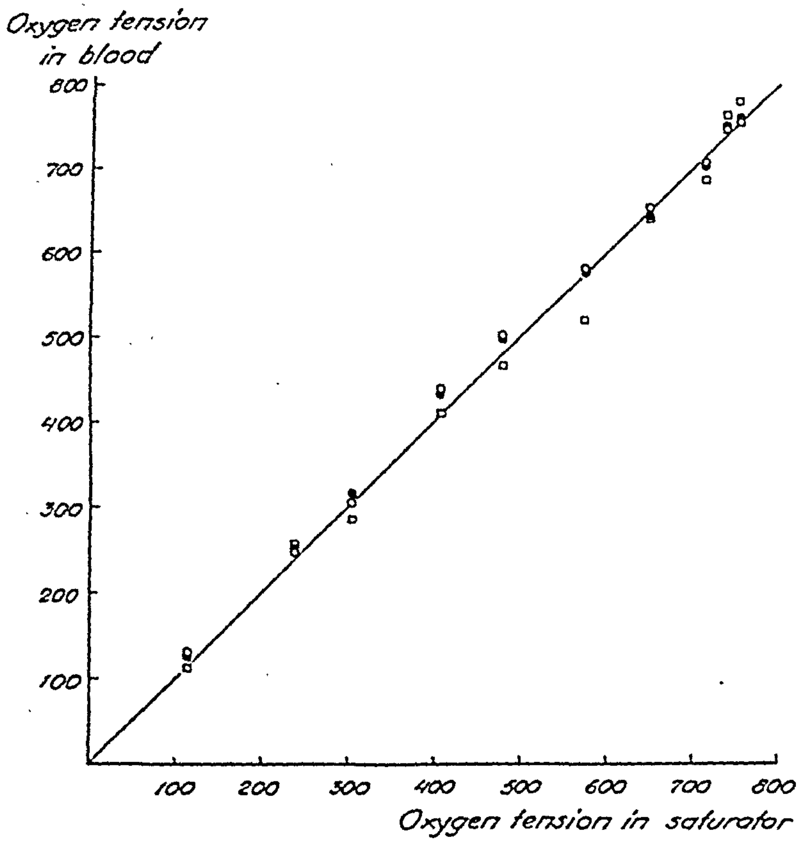


Fig. 10 and Table IV. The connection between oxygen tension of gas in saturator and oxygen tension in plasma determined polarographically and by means of evacuation method.

● oxygen tension by polarograph at —0.7 V.
○ » » » » » —1.6 V.
□ » » » evacuation method.

Table IV.

	Oxygen tension in the liquid phase evaluated by:		
	Polarograph		Evacuation method
	− 0.7 V.	− 1.6 V.	
Coefficient of regression	0.9794 ± 0.0045	0.9770 ± 0.0048	1.0044 ± 0.0113
Mean difference from the tension in the gas-phase (mm.) . .	6.5 ± 3.2	6.1 ± 3.5	− 6.9 ± 7.2

deviation of the calculated oxygen tension from that found in the sample. This is not, however, the case.

The evacuation method has the theoretical advantage of making it possible to isolate the substance to be determined, while in the polarograph only a function of this can be measured, i. e. the diffusion velocity. As a general rule, however, the conditions of the blood affecting the diffusion velocity, especially the content of water and of proteins, are so constant that this function may be regarded as a quantitative measure. Apart from the series of determinations on normal subjects, this is also clear from table V, which shows the results when saturating blood from various patients with a known oxygen mixture. The difference from normal subjects of the values at —0.7 V. is not significant, and the readings at the second level do not differ more than 1 % from the normal values. The determination of the solubility coefficient with the evacuation method gives no significant difference.

In the event of hemolysis a systematic source of error appears in consequence of a tendency to raise the first level of the polarographic curve. This is seen from table VI where samples have been collected in which hemolysis had occurred. The rise of the level makes itself visible in a lower value for the number of mm. oxygen that corresponds to one scale unit of the galvanometer. The table shows that the rise is highly significant. The readings from the second level and the solubility coefficient determined by the evacuation method show no certain difference from the normal values.

By analysing the variation within and between the groups of double tests (BONNIER and TEDIN, 1940) a significant difference appears in some cases (solubility coefficient for patients' blood, the polarographic value at —0.7 V. for normal subjects and at —1.6 V. for both groups). As will be seen when examining the tables, these individual variations are, however, small and have therefore not been considered in the experiments where the oxygen tension has been determined directly on arterial blood. The average values from normal subjects have generally been used when converting from galvanometer deflection and oxygen content to oxygen tension.

In the polarographic method the determination does not change the sample.[1] This makes the method particularly suitable if the

[1] The oxygen consumption due to reduction of oxygen by the electric current can be calculated in the following manner: According to VITEK's conception of the

Table V.

Calibration of patients' blood.

Date		Saturator mm. oxygen tension	Polarograph				Evacuation method	
			Galvanometer deflection		mm. oxygen tension per galv. unit		ml. oxygen per ml. plasma	Solubility coefficient
			−0.7 V.	−1.6 V.	−0.7 V.	−1.6 V.		
23. 2	V.K.	715	27.2	48.7	26.3	14.7	0.0200	0.0212
	(15)[1]	720	27.7	48.8	26.0	14.8	0.0201	0.0212
23. 2	E.K.	710	27.0	48.5	26.3	14.6	0.0196	0.0210
	(3)	725	27.5	49.0	26.4	14.8	0.0199	0.0209
24. 2	E.N.	728		49.3		14.8	0.0204	0.0213
	(12)	737		49.9		14.8	0.0209	0.0216
24. 2	G.P.	733	27.2	48.1	27.0	15.2	0.0202	0.0210
	(1)	733	27.4	48.8	26.8	15.0	0.0201	0.0208
24. 2	K.A.	714	27.0	48.1	26.4	14.8	0.0199	0.0212
	(4)	714	26.7	47.6	26.8	15.0	0.0199	0.0212
24. 2	S. F.	728		48.2		15.1	0.0206	0.0215
	(14)	728		48.3		15.1	0.0204	0.0213
25. 2	H.E.	738		49.8		14.8	0.0205	0.0211
		738		50.0		14.8	0.0209	0.0215
25. 2	C.C.	732		49.8		14.7	0.0199	0.0207
	(2)	732		49.8		14.7	0.0202	0.0210
Mean value					26.50	14.86		0.02116
Standard deviation					0.33	0.17		0.00025
Standard error of the mean					0.12	0.04		0.00006
Difference from normal blood					− 0.20	0.16		− 0.00026
P[2]					>0.1	>0.01		>0.05

oxygen reduction the equivalent weight of oxygen is 16. The solubility coefficient of plasma for oxygen at 37° is 0.0209 and plasma in equilibrium with 1 atmosphere oxygen contains 0.0209:22.4 = 0.000933 gram molecules pr l. i. e. 0.000001866 equivalents pr ml. For reduction of this amount 0.000001866 × 96 494 = 0.18 coulombs are necessary. The polarographically registered current in this sample will be 3.74×10^{-6} amp. For a reduction of 1 % of the dissolved oxygen in a sample of 1 ml. this current must continue for 480 sec. As the polarographic determination takes but 1 min. it is apparent that this consumption of oxygen by the current does not play any rôle as a source of error.

[1] Figure between brackets refers to case histories on pp. 60 ff.

[2] P indicates the degree of probability that the differences are due to mere chance (see BONNIER and TEDIN 1940).

Table VI.

The effect of hemolysis.

Date		Saturator mm. oxygen-tension	Polarograph				Evacuation method	
			Galvanometer deflection		mm. oxygen tension per galv. unit		ml. oxygen per ml. plasma	Solubility coefficient
			− 0.7 V	− 1.6 V	− 0.7 V	− 1.6 V		
20. 2	L. G.	735	28.6	50.1	25.7	14.7	0.0208	0.0215
		755	29.0	51.6	26.0	14.6	0.0213	0.0215
24. 2	E.N.	728	28.5	49.3	25.6	14.8	0.0204	0.0218
		737	28.5	49.9	25.9	14.8	0.0209	0.0216
24. 2	S. F.	728	27.3	48.2	26.7	15.1	0.0206	0.0215
		728	28.4	48.3	25.6	15.1	0.0204	0.0213
25. 2	H.E.	738	28.5	49.8	25.9	14.8	0.0205	0.0211
		738	29.0	50.0	25.4	14.8	0.0209	0.0215
25. 2	C. C.	732	28.0	49.8	26.2	14.7	0.0199	0.0207
		732	29.2	49.8	25.1	14.7	0.0202	0.0210
14. 2	S. B.	725	27.2		26.6			
		725	27.9		26.0			
Mean value					25.89	14.81		0.02130
Standard deviation					0.45	0.17		0.00028
Standard error of the mean . . .					0.13	0.05		0.00009
Difference from normal blood . .					− 0.81	0.11		− 0.00012
P					<0.001	>0.05		>0.05

consumption of oxygen by blood constituents is to be followed. As has been already stated, this is necessary if the determination has to be carried out with plasma at the temperature of the body. Another advantage with the polarograph is that its sources of error are proportionate to the oxygen content, thus making it vastly superior to the evacuation method when low oxygen content is concerned. In the evacuation method the error has an absolute value, which is due to minimal admixtures of gas from the evacuated solutions and to gas extracted from the liquid in the neck of the pipette, which comes into contact with air during the analysis.

Also when working with blood of high oxygen tension as has been the case in this investigation, the polarograph stands above the evacuation method with regard to accuracy as well as to the simplicity with which the analyses are made. With continued con-

trol of the flow of mercury and correction to a fixed drop time, there is no systematic source of error of such magnitude that it can exceed the limits of error already indicated. It is possible to obtain a reliable value from one single determination and double analyses are unnecessary. Concerning the evacuation method on the other hand, as is the case with all methods where gases are extracted, considerable errors must sometimes be reckoned with, owing to the fact that if great care is not taken when manipulating the pipette, a small part of the gas bubble can go out through its neck or air may slip in the same way. It is therefore never possible to draw any conclusion from the single analysis, double ones must be carried out. That these most often give consistent results is obvious from the series presented in tables IV—VI. Sometimes, however, far too low values are obtained.

From certain points of view it is of minor importance which level of the polarographic curve is used for the readings. As was clear from the above mentioned series, the accuracy was practically identical for both levels. The second level has the advantage of being flatter, making slight potential variations of less importance. As was seen in the determinations on hemolyzed plasma, moreover, the second level is not affected by the hemolysis as was the case with the first. On the other hand this is at a satisfactory distance from the potential at which sodium begins to be reduced. The drop time decreases with increasing potential and it seems to be easier to get a constancy of drop time at the lower level. In these investigations the lower level has generally been used for the determination, the reading at the second level only being taken as a control.

On account of the greater accuracy and simplicity of the polarographic method, the evacuation method has from now onwards only been made use of now and again. The principal object for making use of the evacuation method in this work has been for the purpose of controlling the specificity of the polarograph when determining oxygen in blood. By using a larger quantity of blood for the analyses it may perhaps be possible to come to the same accuracy with the evacuation method as with the polarograph, but at least 1 ml. plasma would be necessary. In order to be able to analyze this quantity there must be a corresponding increase in the size of the gas chamber.

If there are considerable supplies of blood, it will be possible to come to a corresponding accuracy by means of analyses in the

ordinary VAN SLYKE-NEILL apparatus with 5—10 ml. plasma. In order to be able to carry out a double analysis 25—50 ml. blood will consequently be required. As the samples should be taken over mercury and as the rapid chilling and the centrifuging at low temperature necessitates special arrangements this method would be rather circumstantial. It may be preferable, however, if there is a VAN SLYKE-NEILL apparatus at hand and its technique thoroughly understood.

Method for the Determination of Admixture of Venous Blood to the Arterialized Blood.

The subject breathes out of a rubber bag attached to an oxygen bomb through a LOVÉN valve with a tightly fitting mouthpiece. Previous to the experiment the bag is flushed a couple of times with oxygen to ensure that there is really 100 % oxygen in the inspired air. The first sample of alveolar air is taken after 12 min. in an ordinary HALDANE pipette in a number of fractions towards the end of normal expirations. About 15 min. after the beginning of the experiment arterial puncture is carried out by means of a needle with an exterior calibre of 0.75—0.9 mm. which is attached to a glass syringe. This has the advantage of being so constructed that the piston moves more easily than in an ordinary record syringe and never gets locked. The dead space of the glass syringe and the needle is filled with a 1—2 % solution of heparin in physiological saline. It is well to make the puncture in the arteria brachialis just above the lacertus fibrosus. The position of the artery is controlled by means of palpation, and to prevent the blood-vessel from slipping it is best to press it against the support. No anæsthesia is necessary when using such a fine needle. As soon as the point comes into the blood-vessel the piston begins to move. The size of the blood-sample is usually 5 ml. As soon as it has been taken, a compression bandage is applied and the sample is transferred as quickly as possible to ice water, where it is kept until the analysis can be made. The experiment concludes with another sample of alveolar air.

The analysis of alveolar air is made in a HALDANE gas analysis apparatus. In order to avoid all risk of an incomplete resorption of the oxygen, it has been found best to confine the size of the sample to 2 ml.; this is mixed in the apparatus with 8 ml. nitrogen, which has previously been carefully measured. The error of the analyses are reckoned at 0.3 %, corresponding to a tension of 2—3 mm.

Before determining the oxygen tension the blood must be well mixed in the syringe by sucking up a little mercury and then shaking. The blood is injected into two of the tubes described along with the polarograph. These have previously been filled with mercury by means of which all contact with the air is avoided. The oxygen tension is then determined polarographically, as has already been described, and an

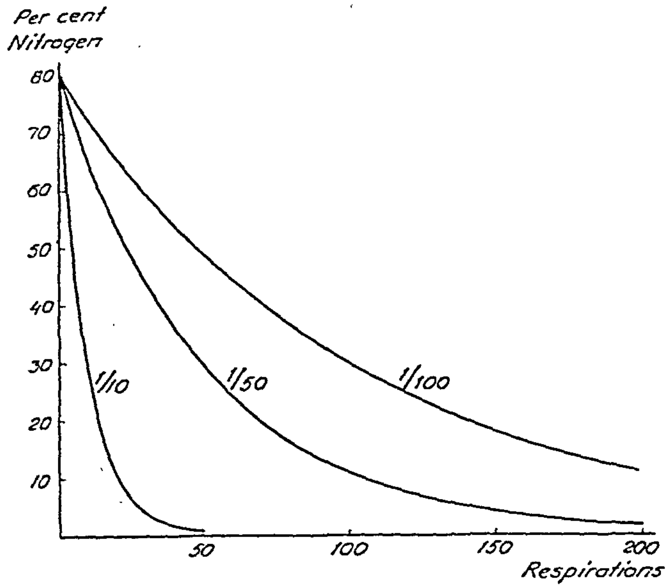


Fig. 11. Influence of degree of ventilation on elimination of nitrogen during oxygen inhalation. The figures attached to each curve give the amount of the alveolar air renewed with each breath.

analysis of the oxygen content of the plasma may possibly be made according to the evacuation method. The occurence of hemolysis is controlled spectroscopically.

In order to be able to compare the oxygen deficit of arterial blood with the oxygen capacity the hemoglobin content has been determined by a Zeiss hemometer, which has been calibrated by comparing its value with the oxygen capacity according to VAN SLYKE.

When calculating the oxygen tension, the dilution of the blood that takes place by means of the heparin solution in the dead space of the syringe must be taken into consideration. When making this correction, the value of the oxygen tension found is multiplied with the degree of dilution, which is determined by weighing out the dead space. The syringe used in these tests had a dead space of 0.12 ml. and as the size of the sample is 5 ml., the degree of dilution will be 1.025. As the heparin solution, however, is not free from oxygen but can be reckoned to be in equilibrium with the oxygen tension of air, a value is subtracted corresponding to its oxygen content. This correction amounts to 6 mm.

The difference of tension has been converted into content and has been expressed in percentage of the oxygen capacity (= percentage oxygen unsaturation). When converting oxygen tension to oxygen content, the solubility coefficient for blood of 0.0230 given by SENDROY, DILLON and VAN SLYKE (1934) is applied.

A necessary condition for this method to determine the admixture of venous blood is that the greatest possible part of the nitrogen is eliminated from the lungs previous to the arterial puncture.

Table VII.

Nitrogen percentage in alveolar air at intervals after the beginning of oxygen inhalation.

	4 min.	12 min.	≥ 17 min.
	2.2	1.0	0.7
	4.6	0	0.6
	3.0	0.4	0.7
	2.8	2.5	0.1
	0.5	1.0	0
	1.1	0.6	0.7
	2.2	1.0	0.7
	2.6	0.8	0.2
Mean value f	2.35	0.91	0.46
Standard deviation	1.28	0.73	0.31
Standard error of the mean	0.45	0.26	0.11

As will be seen from fig. 11, under normal circumstances (residual air + reserve air. c. 3,500 ml., alveolar ventilation 350 ml. per breath) 35—40 breaths are required to reduce the nitrogen content to 2 %. Thus for normal conditions the 15 min. are more than sufficient. In ventilation so strongly reduced that only 1/50 of the alveolar air is renewed with every fresh breath, approximately 200 respirations are required to make the nitrogen content sink to 2 %. With a respiration frequency of 15 per min. this concentration will thus be reached in c. 13 min. Such imperfectly ventilated parts as these mentioned here can only be met with to a limited extent. The existence of a still worse ventilated part of the lung cannot be excluded under certain pathological conditions, e. g., in the case of emphysema. As they cannot, however, be considered very extensive, and, as there is sufficient oxygen in any case to entirely saturate the hemoglobin, they are not calculated to be any great source of error. In order to prove the correctness of the above calculations, some tests are shown in table VII, where an additional alveolar sample was taken after inhaling oxygen for 4 min. As will be seen most of the nitrogen will have been eliminated by this time. Furthermore, a comparison between the values just before and just after the arterial puncture shows a difference generally amounting to less than 1 %.

When taking a sample of alveolar air in the manner described,

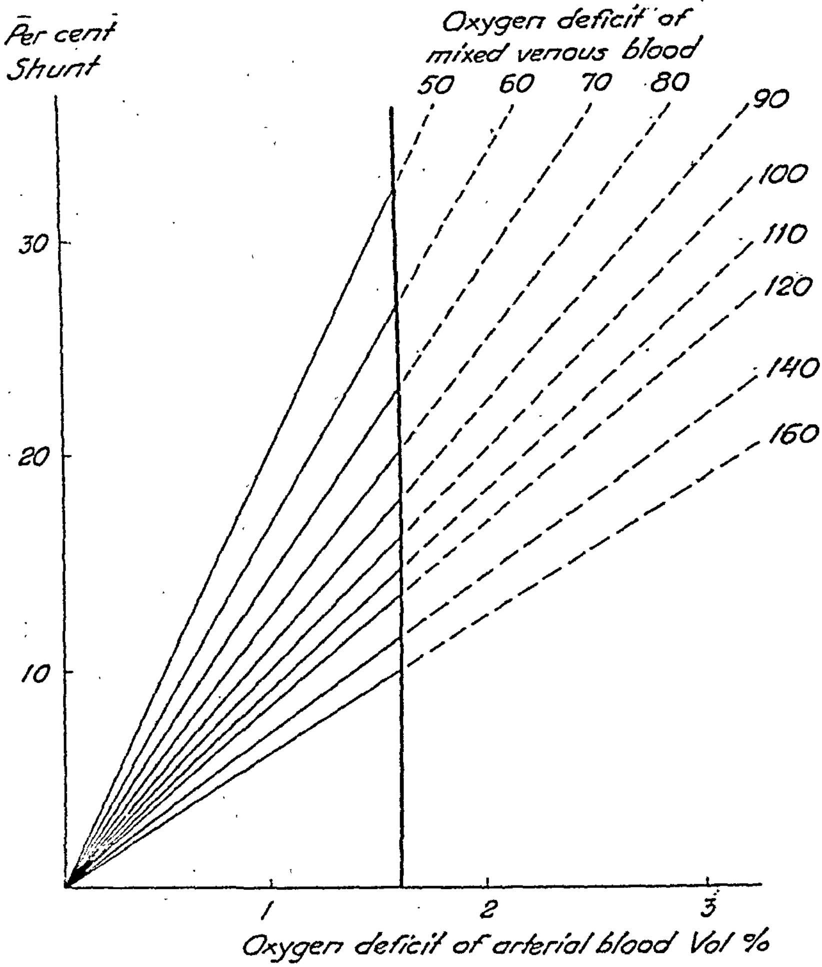


Fig. 12. Influence of variations in oxygen deficit of venous blood on arterial oxygen deficit caused by varying amounts of blood, short-circuited past ventilating alveoli (% shunt). Left of perpendicular line hemoglobin will be completely oxygenated when breathing pure oxygen and deficit of arterial blood can be determined by analysing physically dissolved oxygen in plasma after centrifuging. Oxygen deficit of venous blood is expressed in ml pr l.

the amount of tidal air may be too small to completely wash out the dead space. Considering the values of carbon dioxide, they are often under 5 %, while the normal content in the alveoli is c. 5.5 %. The error thus arising is most probably less than it would have been if the patients had tried without careful training to make such deep expirations as are necessary when taking samples of the alveolar air according to the original Haldane technique (cp. HASSELBALCH and LINDHARD 1911).

The deficit of oxygen found in the arterial blood is an expression

for a blood-flow through non-ventilated parts of the lung. In order to determine the total flow passing through similar parts of the lung, it is necessary also to know the oxygen deficit of the venous blood. The greater this is, the greater will be the decrease in the oxygen content of the arterial blood arising through the admixture of venous blood. The diagram in fig. 12 shows the relation of some different values of the venous oxygen deficit to this admixture. It is also expressed by the following formula:

$$x \cdot v = a$$

(x = non-arterialized part of blood-flow. v = oxygen deficit of venous blood. a = oxygen deficit of arterial blood). The deficit refers to the oxygen content of the fully arterialized blood, i. e. blood in tension equilibrium with alveolar air.

The perpendicular line on the diagram represents the limit for the method given. As will be seen by the tests on patients with pulmonary diseases, this limit is hardly ever exceeded. The values beyond this (i. e. the surplus of physically dissolved oxygen is not sufficient to fully saturate the hemoglobin of the admixed venous blood) are accessible, if an ordinary analysis is made according to VAN SLYKE.

When experimenting with animals, the deficit of venous blood is easily obtained by an oxygen determination in blood from the right auricle or ventricle. In man it can be obtained by a determination of the arterio-venous oxygen difference according to GROLLMAN's (1932) acetylene method. In its original form, however, this method is unsatisfactory when experimenting on pathological cases, and in order to form a conception of the arterio-venous oxygen difference in these, circumstantial modifications are necessary (NIELSEN 1937, BERSÉUS 1942). No attempts have been made to carry out similar determinations, but values in various cases of illness obtainable from literature have been used when discussing the respective cases. With the acetylene method the oxygen absorbed by the blood passing ventilated alveoli is determined. As this blood comes into complete equilibrium with the alveolar air, the arterio-venous oxygen difference calculated from this method is equal to the oxygen deficit of venous blood and can be used for v in the formula given above.

APPLICATIONS.

Determinations of the Admixture of Venous Blood on Healthy Subjects.

Table VIII gives a survey of a series of determinations made on healthy subjects of the oxygen tension difference between alveolar air and arterial blood during oxygen inhalation. The subject was sitting in a deck-chair and there was a short rest of 5 min. previous to the test. Apart from this no other standard conditions have been followed.

A slight deficit of oxygen in the arterial blood compared with the alveolar sample is regularly obtained. The carbon dioxide content of the alveolar air is in the majority of cases above 5 %, and therefore it may be right to assume that the values of oxygen tension in the alveolar sample correspond to the true alveolar air.

When breathing ordinary air a slight tension difference up to 30 mm. is regularly obtained in man (Barcroft, Cooke et al. 1920, Bock, Dill et al. 1929, Dill, Edwards et al. 1931, Kroetz 1931, Kramer and Sarre 1935, Dill, Christensen et al. 1936, Matthes et al. 1936, Brinkman and Dirken 1940). While certain authors (Krogh 1910, Kroetz 1931, Sarre 1935) in the first place regard this difference as being an expression for the fact that the diffusion through the alveolar epithelium is not complete, others maintain that it is due to the existence of an irregular ventilation (Haldane and Priestley 1935) or to the admixture of venous blood from the bronchial veins (Knipping 1935). As has already been analyzed in an earlier chapter (p. 11) the first explanation is unlikely, the size of the diffusion constant being sufficient to prevent the tension difference from exceeding a fraction of a mm.

Bock, Dill, Edwards, Henderson and Talbott (1929) found that even when breathing air of high oxygen content, complete saturation of the hemoglobin could not be obtained, which means a

Table VIII.

Difference in oxygen tension between alveolar air and arterial blood: Normal subjects.

Case		Alveolar air % CO_2	Alveolar air % O_2	Alveolar air mm. oxygen tension	Alveolar air mm. oxygen tension Mean	Arterial blood mm. oxygen tension	Arterial blood mm. oxygen tension Mean	Oxygen tension diff. mm.	Oxygen deficit ml. O_2 pr ml. blood	Oxygen capacity Vol.%	Oxygen deficit % unsaturation
1	S.B.				645		635	10	0.00030	18.6	0.16
2	R.B.	5.3	94.0	658	660	640	643	17	0.00051	18.8	0.27
		5.3	94.6	662		645					
3	Å. H.	4.5	93.3	653	662	642	642	20	0.00060	19.4	0.31
		4.4	95.6	670		642					
4	N. R.	4.9	93.7	658	658	651	652	6	0.00018	16.7	0.11
		4.6	93.8	658		653					
5	J. H.	4.0	93.7	658	664	648	650	14	0.00042	16.7	0.25
		4.1	95.5	670		651					
6	O. P.	5.5	94.6	664	665	659	656	9	0.00027	17.1	0.16
		5.2	94.8	666		653					
7	H. M.	4.8	94.7	664	666	653	656	10	0.00030	17.9	0.17
		5.0	95.0	667		659					
8	S.K.	5.0	93.7	658	659	642	646	13	0.00039	17.7	0.22
		5.3	93.9	659		648					
9	B.L.	5.1	90.0	663	680	676	679	1	0.00003	17.8	0.02
		5.0	94.3	696		681					
10	A.B.	5.5	92.1	680	680	667	669	11	0.00033	17.1	0.19
		5.3	92.1	680		670					
							Mean	11.3 ± 1.5			0.186

greatly increased tension difference. They explained this by assuming that the hemoglobin molecules most distant from the surface of the blood-cells have not time to absorb oxygen during the passage of the blood through the capillaries of the lung, and that equilibrium is not brought about until the blood has passed by the capillaries. MATTHES, GIBERT QUERALTO and MALIKIOSIS (1937) maintained that complete saturation of the hemoglobin is only reached at an alveolar oxygen tension of 300 mm. and this implies an oxygen tension difference of 130 mm.

These last mentioned investigations are not in agreement with those presented here. BOCK and collaborators determined the oxygen tension of the blood indirectly from the hemoglobin dissociation curve and the degree of oxygen saturation according to VAN SLYKE. The accuracy of this method within the sphere of almost complete saturation is not satisfactory owing to the fact that a fairly large addition to tension causes a slight addition to the degree of saturation. Whether the diffusion of oxygen to paraffin or the actual oxygen consumption of the blood has been able to cause a decrease of the oxygen content of the samples, is not clear from the technical statements given. MATTHES et al. made their determination by means of absorption photometry on an histaminized lobe of the ear. There is not sufficient support to prove the absence of oxygen consumption in the tissues under their experimental conditions, and furthermore it seems doubtful whether the photometric accuracy can be carried as far as the authors consider to be the case.

In consequence of the conditions under which the experiments have been carried out, irregular ventilation or impaired diffusion cannot have affected the results presented. As the tension difference established is due to an admixture of non-arterialized blood, the tests show that the size of this admixture is small under normal conditions. Without being obliged to assume the existence of any occluded parts of the lung in healthy subjects, this small admixture of non-arterialized blood may be explained anatomically by the fact that the bronchial veins partly empty themselves into the pulmonary (MILLER 1906, 1925, MATHES et al. 1932) or else because of the existence of arterio-venous anastomoses (HAYEK 1940). Assuming an arterio-venous oxygen difference of 60 ml. pr l. (GROLLMAN 1932) this admixture of venous blood does not exceed 0.6 % of the total blood-flow through the lung.

Apart from the question whether the tension difference found really is a significative expression for a shunting of blood past ventilating alveoli, this series seems to represent a decided proof that no change takes place in the physically dissolved oxygen in plasma by chilling the blood to zero or by centrifuging. If really an adsorption process should appear in vitro, as SPEITKAMP found (1939), this small difference in oxygen tension between alveolar air and arterial blood would be inconceivable.

Table IX.

Difference in oxygen tension between alveolar air and arterial blood. Patients confined to bed but pulmonarily healthy.

Case		Age	Alveolar air % CO_2	Alveolar air % O_2	Alveolar air mm. oxygen tension	Mean	Arterial blood mm. oxygen tension	Mean	Oxygen tension diff. mm.	Oxygen deficit ml. O_2 pr ml. blood	Oxygen capacity Vol.%	Oxygen deficit % unsaturation
1	S.	56	4.9	95.1	671		623 628	626	45	0.00136	17.1	0.80
2	B.	41	5.2 4.6	93.9 95.4	661 671	666	661 650	656	10	0.00030	19.8	0.16
3	H.	41	3.8 3.9	95.3 96.1	672 677	675	617 620	619	56	0.00170	18.6	0.91
4	M.	40	5.2 5.2	94.8 94.0	667 662	665	606 601	604	61	0.00185	18.6	0.99
5	A.	28	4.8 4.9	94.3 94.2	660 660	660	650 647	649	11	0.00033	18.4	0.18
6	S.	46	4.8	93.9	657		650 645	648	9	0.00027	18.0	0.15
7	P.	37	4.9 4.6	94.1 94.7	661 664	663	615 626	621	42	0.00127	17.7	0.72
8	A.	54	4.4 4.0	93.5 95.8	688 705	697	653 653	653	44	0.00133	17.2	0.77
9	J.	49	4.8 5.0	95.3 93.9	668 659	664	574 562	568	96	0.00290	16.7	1.74

Determinations on Patients Confined to Bed.

Table IX shows a number of determinations carried out on patients confined to bed but pulmonarily healthy. As will be seen, the tension difference obtained in several cases is considerably higher than was found in normal subjects. The explanation of this increased tension difference may be attributed to the clinically familiar signs of atelectasis in the lower part of the lung, which appear in the form of fine râles, when a patient in bed takes a deep breath. The existence of arterio-venous anastomoses

(HAYEK 1940) may be another explanation, as through these channels the blood in an increased flow may pass the capillaries in the alveoli during the complete rest that the lying in bed brings about.

Case 9 showing the greatest difference in the group deserves special notice. The patient in question had a moderate arcuate kyphosis in the upper part of the thoracic spine. It seems probable that such a deformation of the thorax intensifies the tendency to hypo-ventilation in the lower parts of the lungs, which is usually looked upon as a predisposition to atelectasis (cp. the discussion on the causes of post-operative atelectasis: CORYLLOS and BIRNBAUM 1928 and others).

Determinations on Patients with Pulmonary Diseases.

The existence of occluded parts of the lung is common in pulmonary pathology. Distinction must here be made between processes where occlusion from ventilation is brought about by infiltration in the parenchyma or exudation in the alveolus (e. g. pneumonia) on the one hand and the different types of atelectasis through obturation of a bronchus or external compression by means of e. g. exudation or pneumothorax on the other. A number of investigations on the oxygen content of arterial blood have been carried out in these conditions, only a small number of which, however, clearly illustrate the question of the blood-flow through the occluded parts.

In a disease in which the occluded parts are greatly extended, such as pneumonia or acute massive atelectasis, the blood-flow through these parts may be expected to be so great that the deficiency of arterial saturation thus arising dominates the other causes. The great deficiency occurring in certain cases of pneumonia has also been interpreted in this manner (HOOVER 1918, STADIE 1919, BINGER 1928/29), but by other investigators anoxæmia has been explained as a result of shallow breathing (MEAKINS and DAVIES 1921) or else due to an existing pulmonary œdem (BARACH and WOODWELL 1921). For the correctness of the last mentioned opinions the effect of oxygen therapy, inter alia, has been referred to, which often causes the complete disappearance of the anoxæmia.

In other diseases in which there are presumed to be non-ven-

tilated parts of the lung, such as pneumothorax, thoracoplasty or pleurisy, the result of the determinations of oxygen saturation varies considerably from case to case and with different investigators. While MEAKINS and DAVIES (1925) maintained that a deficit of oxygen often disappears when pneumothorax has been established owing to the fact that badly ventilated parts then collapse and the blood-flow lessens, LORENZ and WÜLLENWEBER (1932) considered that there is a regular increase in the deficit after double pneumothorax has been established. Other authors, e. g. HILTON (1925) found now a normal, now a reduced saturation and did not consider that there is any connection between the degree of compression and the pneumothorax age and an existing deficit. While HILTON considered that the deficiency of saturation is due to a short-circuit of blood through non-ventilated parts of the lung, TÖRNING (1933) supported by experimental tests considered that it is due to an impaired oxygen absorption in the free lung.

With the method above described, it is possible to isolate one of these causes, i. e. the blood-flow through non-ventilated parts of the lung. In order to find out the applicability of the method for this clinical problem, determinations have been carried out on a number of patients with manifest changes in the lungs, especially cases where there has been reason to presume the existence of atelectases by obturation or compression.

Atelectasis through Pleural Exudate and Pneumothorax.

Case 1. Göte P. 16 years. *Serofibrinous Pleurisy.* Taken ill acutely 24. 1. 42. with fits of shivering and pain in the side of the chest. After 31. 1. the pain disappeared, but he felt short of breath. On admission to hospital 2. 2. a thoracocentesis was made on the right side, 1,700 ml. exudate were aspirated and 600 ml. air was insufflated. An X-ray 3. 2. showed a dense shadow over the basal parts of the right lung with a slight displacement of mediastinum to the left and a moderate pneumothorax (fig. 13).

Temperature (rectal) 3. 2. 38.3—38.9. Sedimentation rate of erythrocytes 13 mm./hr.

3. 2. 42. Arterial oxygen deficit during oxygen inhalation 285 mm., corresponding to 5.3 % unsaturation. A new determination 4. 2. gave the values 325 mm. and 6.2 % saturation respectively. Arterial oxygen content during inhalation of air, 15.1, 15.2 vol.%, oxygen capacity 16.8, 16.7 vol.%, oxygen saturation 90.5 %.

17. 2. The arterial oxygen deficit during oxygen inhalation has diminished to 176 mm., corresponding to 3.5 % saturation. The patient

was then afebrile. Sedimentation rate 95 mm./hr. In the meantime 2,800 ml. exudate was aspirated.

In this case with a considerable exudation on the right side of recent date, a compression of the greater part of the lower lobe on this side may be expected. The extent of the atelectatic parts will probably amount to 1/4 at most of the total parenchyma of the lung. The oxygen deficit found while breathing oxygen in determinations taken on two consecutive days is 285 and 325 mm. respectively, figures that the mere fact of lying in bed cannot explain. Assuming a normal arterio-venous oxygen difference of 60 ml. pr l. [According to investigations by inter alia NYLIN (1933) and BLUHM (1935), the cardiac output is unchanged when one lung is compressed by pneumothorax, and therefore there is no reason to expect any great deviation from the normal arterio-venous difference. This does not seem to be greatly affected either by fever (BJERLÖV and LILJESTRAND 1927, GROLLMAN 1932) or by the patient being examined in a recumbent position (GROLLMAN 1932)], the blood-flow through the atelectatic area will amount to c. 15 % of the total. Three weeks later, during which time exudate had been repeatedly aspirated, the deficit sank to half, an expression for a reduction in the size of the atelectasis or its blood-flow.

Case 2. Carola C. 65 years. *Hyper-tension + Cardiac Incompensation with Pleural Transudation.* Taken ill in the middle of Jan. 1942 with cough, a slight temperature and dyspnoe. On admission to hospital a decreased resonance was found over both bases of the lungs with weakened breath sounds. X-ray 3. 2. Dense shadows basally within both lung areas with a laterally rising border. Central vascular congestion, heart enlarged (fig. 14).

5. 3. 42. Arterial oxygen deficit during oxygen inhalation 317 mm., corresponding to 5.0 % unsaturation.

The decreased resonance over the bases of the lungs quickly disappeared, on 13. 2. the resonance was normal. A second determination of the arterial oxygen deficit during oxygen inhalation on 25. 2. showed a decrease to 43 mm. or 0.7 % saturation.

At the time of the examination there was transudation in both pleural cavities as the most important manifestation of a cardiac incompensation, which had developed in the course of a few weeks, thus, in this case as well, fairly fresh changes. The examination showed a considerable deficit (317 mm.), corresponding to a blood-flow of 10—15 %. Under hospital treatment the transudates disappeared in about a fortnight, and when determining the oxygen

deficit under oxygen breathing one week later, the deficit had practically speaking disappeared and was not higher than could be attributed solely to the lying in bed.

Case 3. Erik K. 44 years. *Serofibrinous Pleurisy + Pneumothorax.* Taken ill acutely at the beginning of Sept. 1941. Roentgen examination showed a dense shadow on the left side with a rather pronounced displacement of the mediastinum. Thoracocentesis with aspiration of fluid 1—2 times a month, the last time (30. 1. 42) 650 ml. Roentgen examination 13. 1. showed an exudate to the level of the eighth rib and collapse of the left lung with only a narrow layer of lung tissue visible close to the mediastinum. Moderate displacement of the mediastinum. (fig. 15).

Afebrile for several months. Sedimentation rate 30 mm.hr.

3. 2. 42 Arterial oxygen deficit during oxygen inhalation 78 mm., corresponding to 1.5 % unsaturation. Repeating the determination on 23. 2. gave 89 mm. or 1.7 % unsaturation. In the meantime aspiration of 1,550 ml. exudate in all. Roentgen examination on 7. 3. showed a partial re-expansion of the lung.

This is a case of long standing pleurisy, in which the lung was practically entirely collapsed for several months owing to exudation and pneumothorax. In spite of the fact that this lung is probably completely shut off from ventilation the oxygen deficit is very small (78 and 89 mm. respectively, corresponding to a blood-flow of c. 4 %), which proves that the flow through this lung is extremely slight. This case is a striking contrast to the two previous ones, both of which, with a smaller atelectasis, had a considerably greater deficit, and clearly shows the relation between the age of the atelectasis and its blood-flow. The pressure on the lung exerted by the exudate and the pneumothorax, which had led to a displacement of the mediastinum may also be of some importance for the reduction of the blood-flow.

Case 4. Kerstin A. 18 years. *Pulmonary Tuberculosis + Pneumothorax + Empyema.* Tuberculosis of the right lung was diagnosed at the beginning of 1939. Pneumothorax was established June 1939 and later (June 1940) an adherence cauterization was made according to JACOBÆUS. In Dec. 1941 rise of temperature and pains in the chest. Roentgen examination 12. 12. 41. Right lung completely collapsed. Exudate basally in the pleural cavity (fig. 16). 30. 12. 41. Thoracocentesis with aspiration of 900 ml. thick, yellow pus. During Jan. 1942 c. 500 ml. pus was aspirated by thoracocentesis once a week.

Temperature 37.1—37.9 Sedimentation rate 106 mm/hr.

4. 2. 42. Arterial oxygen deficit during oxygen inhalation 181 mm., corresponding to 5 % unsaturation.

In this case, too, there is a complete collapse of the left lung, which had existed for many months. Compared with the degree of collapse, the oxygen deficit is small, though considerably greater than in the previous case. When attempting to convert the oxygen deficit into blood-flow, it must be remembered that the anemia in this case has probably caused an increase in the cardiac output with decreased arterio-venous difference (LILJESTRAND and STENSTRÖM 1925, NIELSEN 1934). The blood-flow can be reckoned as being between 10 and 15 %, which means a decrease to c. 1/3—1/4 of the blood-flow through the left lung compared with a normal state.

Case 5. Göte N. 20 years. *Mediastinal Tumor + Artificial Pneumothorax.* Difficulties in swallowing since June 1941. On 22. 1. 42 pneumothorax was established on the right side for the purpose of diagnosis, the last filling being made on 5. 2. (700 ml. air, final pressure — 5 + 3 cm. water). Roentgen examination showed a tumor-like growth in the mediastinum, just above the diaphragm and not connected with the lung. Thoracoscopy on 30. 1. showed a yellow protrusion in the lower posterior part of the mediastinum (dermoid cyst). The lower lobe of the right lung was partly atelectatic.

6. 2. 42. Arterial oxygen deficit during oxygen inhalation 291 mm., corresponding to 5.4 % unsaturation.

In this case pneumothorax has probably brought about an extensive collapse and a thoracoscopic examination has established the existence of fully developed atelectasis of the lower lobe. The determination shows a considerable oxygen deficit, though not so great as might have been expected if the blood-flow of the lung had been unchanged. Assuming en arterio-venous oxygen difference of 60 ml./l. the bloo.-flow through the atelectatic parts is calculated to 15 % of the total.

Case 6. Ingrid H. 20 years. *Pulmonary tuberculosis + Pneumothorax* April 1941 Erythema nodosum. Roentgen examination 30. 4. 41. Stripes and spotted shadows in the first and second interstitium of the ribs. 24. 1. 42. The shadow had increased in size, centrally a rarefaction was seen. Pneumothorax was established on the right side on 16. 2. 42. Between 17. 2.—12. 3. 42. 8 re-fillings of 350—500 ml. were made, the last day 400 ml. to a final pressure of — 2 + 3 cm. water. Roentgen examination 9. 3. 42. showed a pneumothorax embracing and compressing the whole of the right lung. Adherences to the upper lobe. The lower lobe atelectatic (fig. 17).

Afebrile. Sedimentation rate 4 mm/hr.

13. 3. 42. Arterial oxygen deficit during inhalation of oxygen 365 mm., corresponding to 6.9 % unsaturation.

Thoracoscopy in connection with adherence cauterization a few hours later showed a complete atelectasis of the lower lobe.

This is a case of pneumothorax too, but is of longer standing than the previous one. The last fillings ended in positive expiratory pressure. The oxygen deficit in this case was slightly greater than in the aforenamed, and there was reason to suppose that fairly large atelectatic areas existed. This was verified some hours after the determination by thoracoscopy, when it was found that the lobe was completely atelectatic, which will also be seen from the roentgen plate. With an arterio-venous oxygen difference of 60 ml./l. the deficit corresponds to a blood-flow through atelectatic parts of 19 % of the total. Besides the atelectatic lower lobe there are probably also such areas in the middle and upper lobe, the total amount of atelectatasis thus being estimated to $^2/_3$ of the right lung, which means that the blood-floow through these areas is decreased by c. 50 %.

Case 7. Eva K. 13 years. *Lymphadenoma of pulmonary hilum + Atelectasis + Pneumothorax.* Erythema nodosum at the beginning of Jan. 1942. Roentgen examination showed an adenoma of the left hilum and a shadow in the parenchyma, partly due to atelectasis, within the left upper lobe. Pneumothorax was established on 9. 2. 42. Between 10. 2. and 16. 3. 8 re-fillings of 300—400 ml. air, the last time to a final pressure amounting to —1 + 4 cm. water. Roentgen examination on 17. 3. showed a pneumothorax which embraced the lung from the front as well as from the back. The apex of the lung adhered to the wall of thorax. The atelectatic part of the upper lobe stood out with increased shade (fig. 18 a).

Afebrile. Sedimentation rate 19 mm/hr.

17. 3. The arterial oxygen deficit during oxygen inhalation 120 mm., corresponding to 2.5 % saturation of the hemoglobin.

1. 4. Thoracoscopy + Adherence cauterization. The upper lobe is seen to be completely atelectatic. 2. 4. last refilling 200 ml., final pressure — 4 + 4 cm. Rise of temperature first few days after cauterization. 7. 4. Roentgen examination: Complete collapse of left lung. Mediastinum somewhat displaced to the right. Slight pleural effusion (fig. 18 b).

8. 4. 42. Arterio-venous oxygen difference during oxygen inhalation 304 mm., corresponding to 6.3 % unsaturation.

This pneumothorax is somewhat older than the previous case and was carried through about equally intensely, i. e. to positive expiratory pressure after the fillings. The atelectatic part in the upper lobe corresponded to the atelectasis that was found at the first Roentgen examination. This area was not so widespread

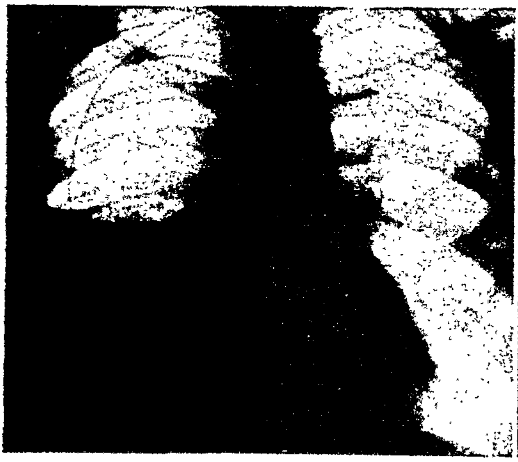


Fig. 13. Case 1. Serofibrinous pleurisy.

Fig. 14. Case 2. Cardiac incompensation with transudation.

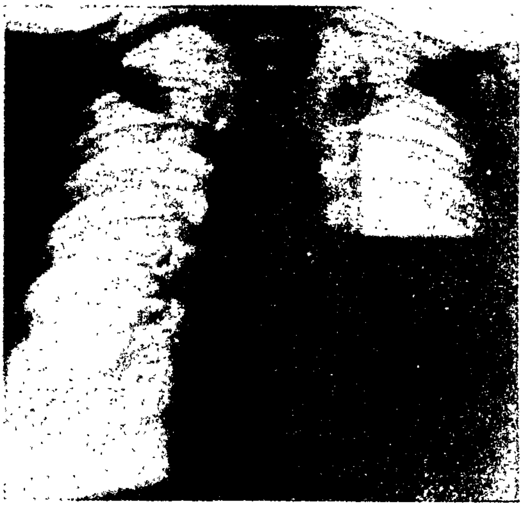


Fig. 15. Case 3. Serofibrinous pleurisy with pneumothorax.

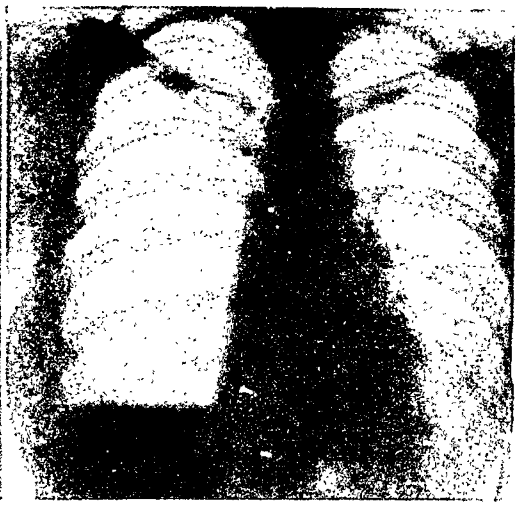


Fig. 16. Case 4. Pneumothorax with empyema.

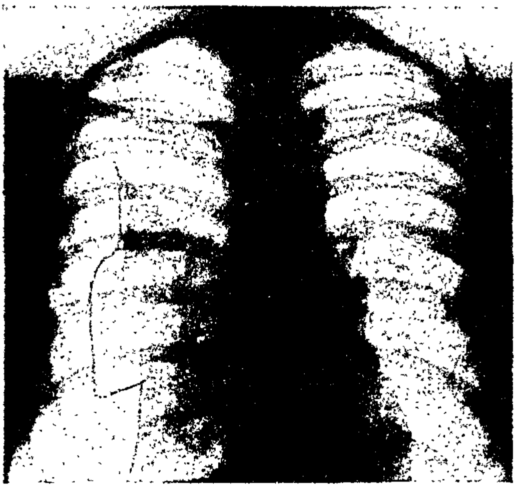
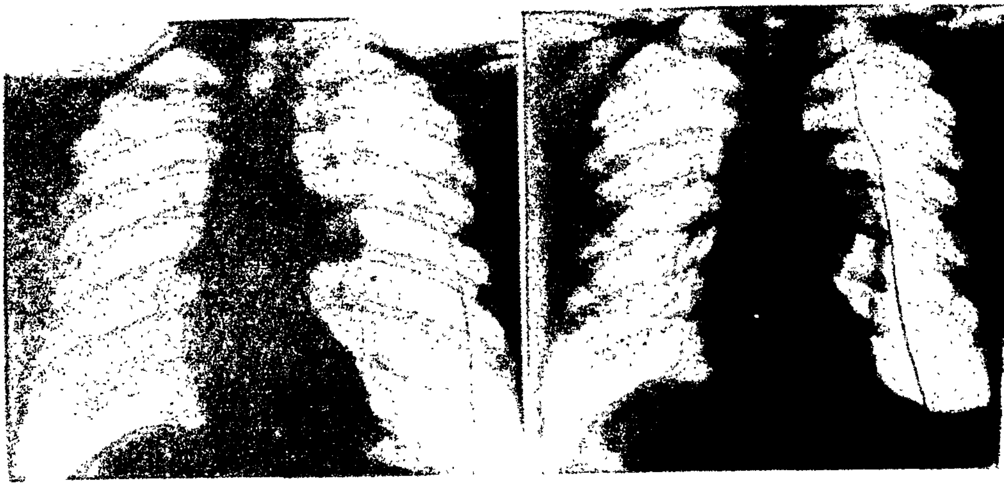


Fig. 17. Case 6. Pneumothorax dxt. Atelectasis of lower lobe.



18 a. 18 b.

Fig. 18 a. Case 7. Pneumothorax sin. Atelectasis of upper lobe.
b. » » Complete collapse of left lung.

Fig. 19. Case 10. Pulmonary tuberculosis with atelectasis.

Fig. 20. Case 11. Post-operative atelectasis.

and was, moreover, older than the atelectasis in the previous case, and these factors will probably explain the much smaller arterial oxygen deficit. As regards age, development, localisation and genesis, this is a complete parallel to case 9, apart from there being no pneumothorax in the latter.

Another examination 3 weeks later showed a considerable rise in the arterial oxygen deficit. This corresponded to the increased degree of collapse shown by the Roentgen plate. Assuming an arterio-venous oxygen difference of 50 ml/l., the deficit indicates a blood-flow through the atelectatic lung of c. 18 %, thus a decrease by a good 50 %. As shown by the first examination the blood-flow through the atelectatic area was rather small. The atelectasis which developed between the examinations probably amounted to more than half of the parenchyma of the left lung. If the blood-flow through the old atelectatic area is considered to be unchanged, the flow through the fresh area will be c. 11 % which is a rather low value when compared with other cases of atelectasis of the same age. As in case 3 this may be due to the high pressure in the pleural cavity, causing displacement of the mediastinum.

Atelectasis through Bronchial Occlusion.

Case 8. Karl S. 61 years. *Bronchial Carcinoma + Atelectasis.* Distressing cough since autumn 1941. Weight dropped by 8 kg. within one year. Roentgen examination 4. 3. 42. Atelectasis of the right upper lobe, especially within its anterior and basal-lateral parts. The right hilum drawn considerably upwards. Trachea and œsophagus displaced to the right. The main bronchus to the right lung is considerably narrowed throughout, and the bronchus to the upper lobe invisible. Bronchoscopy on 5. 3. showed a granulated, easily bleeding tumor at the point where the bronchus to the upper lobe begins.

16. 3. 42. Arterial oxygen deficit during oxygen inhalation 86 mm., corresponding to 1.6 % unsaturation.

It is not possible to give the exact age of this atelectasis, but it is probably several months. The arterial oxygen deficit during oxygen breathing appears to be relatively small as compared with the size of the atelectasis, and should then support the theory of the considerably reduced blood-flow through an old atelectatic area.

Case 9. Ingrid E. 14 years. *Lymphadenoma of pulmonary hilum + Atelectasis.* Taken ill acutely on 24. 1. 42. with fits of shivering, pains in the side of the chest and coughing. Roentgen examination 30. 1. 42

showed a large infiltration of the parenchyma in the posterior part of the upper lobe with a sharp lower border and a pleuritic shadow over the basal parts. 9. 3. 42. The left-sided infiltration of the parenchyma remained unchanged. A considerable atelectasis existed in the left lung with pendulum movements of mediastinum and heart. In the left hilum there was an adenoma, compressing the bronchial branches. There was still a slight exudate in the pleura.

Afebrile. Sedimentation rate 44 mm./hr.

16. 3. 42. Arterial oxygen deficit during inhalation of oxygen 54 mm., corresponding to 1.1 % unsaturation (patient out of bed part of the day; sitting in deck-chair during test).

The age of this atelectasis dates back to the days when the patient was taken ill, i. e. 7 weeks before the determination. The oxygen deficit during oxygen breathing seems small considering the age and size of the atelectasis, which may possibly have something to do with the existence of an inflammatory process within the atelectatic area. This is probably of the type of a perifocal œdem round a tuberculous primary infection. Cp. with case 7.

Case 10. Viola K. 31 years. *Pulmonary Tuberculosis + Atelectasis + Bronchopneumoniae?.* Pulmonary tuberculosis was diagnosed 1922. Visited sanatorium repeatedly. Taken ill acutely on 12. 1. 42 with temperature rising to 39°, pains in the left side of the back and dyspnoe. On admission to hospital 14. 1. 42 there was decreased resonance over the left lung with weak bronchial breath sound, which was all but inaudible below angulus scapulae. Roentgen examinations on 16. and 19. Jan. showed a dense shadow over the left lung which contained air only in its apical parts. Mediastinum displaced to the left, diaphragm was drawn upwards. There was probably a considerable fibrosis of the lung and some atelectasis (fig. 19).

Afebrile. Sedimentation rate 45 mm./hr.

3. 2. 42. Arterial oxygen deficit during inhalation of oxygen 208 mm., corresponding to 4.3 % unsaturation.

23. 2. A second determination showed a decrease of the deficit to 127 mm., corresponding to 2.6 % unsaturation. Roentgen examination showed an unchanged picture. Afebrile. Sedimentation rate 22 mm./hr.

Neither is this case purely atelectatic, but behind the roentgenologically dense shadow there is probably also a bronchopneumonic process. The oxygen deficit found at the first determination corresponds to a decrease in the blood-flow of the diseased parts to c. $^1/_3$ of the normal. As there was roentgenologically no clearing up of the dense shadow, the diminished deficit found 3 weeks later points to a successive decrease of the blood-flow.

Case 11. Sven H. 16 years. *Post-operative Atelectasis.* 29. 3. 42. Appendectomy in ether narcosis. 31. 3. Temperature rising, pains in the

left side of the chest. Weak, bronchial breath sound over the base of left lung with some small râles, no decreased resonance. Roentgen examination: Left diafragm stood high. Diffuse shadow over left base of the lung reaching to the fourth interstitium (fig. 20). 1. 4. Temperature 39.1° C. The bronchial breath sound was heard over the entire lower lobe.

1. 4. 42. Arterial oxygen deficit during oxygen inhalation 380 mm., corresponding to 6.6 % unsaturation.

2. 4. Temperature 37.8 Physical examination of the lungs showed no change. Arterial oxygen deficit during oxygen inhalation 206 mm., corresponding to 3.5 % unsaturation.

4. 4. Temperature 36.9. Sedimentation rate 65 mm./hr. Roentgen examination: The shadow had completely vanished and the level of diafragm was normal. Physical examination showed some râles basally. The breath sounds were somewhat harsher on the left side but not bronchial in character. Arterial oxygen deficit during oxygen inhalation 34 mm., corresponding to 0.6 % unsaturation.

This case must be regarded as a typical post-operative atelectasis of the left lower lobe in which a pneumonic process developed. The oxygen deficit caused by the blood-flow through this area was rather high at the first examination and assuming a normal arterio-venous oxygen difference of 60 ml./l. corresponded to a blood-flow through the atelectatic and pneumonic area which amounted to c. 20 % of the total. As the volume of the affected lobe constituted c. 25 % of the total lung volume the decrease in blood-flow was small. The decrease of the deficit to half on the next day may correspond to a decrease in blood-flow through the consolidated areas. In the genuine lobar pneumonia this successive decrease of the blood-flow through the consolidated areas seems to be a rather regular process as seen from the decrease in arterial oxygen deficit or from the impairment to injection of the vessels (for discussion see Coryllos and Birnbaum 1929). —After two more days all physical and roentgenological signs of consolidation had disappeared and the all but normal arterial oxygen deficit is thus in agreement.

Thoracoplasty.

Case 12. Erik N. 61 years. *Pulmonary Tuberculosis + Thoracoplasty.* In 1939 a cavity was found in the right apical region. Thoracoplasty was performed with resection of nine ribs on the right side. Roentgen examination on 31. 1. 42. The upper two thirds of the left side of the thorax showed a satisfactory collapse, diffuse shadow over the lung area. There had been no change in the roentgen picture since the operation.

5. 2. 42. Arterial oxygen deficit during oxygen inhalation 120 mm., corresponding to 2.0 % unsaturation.

Table

Pathological

Case	Date	Name Diagnosis	Alveolar air				Arterial blood	
			% CO_2	% O_2	mm. Oxygen tension		Polarograph − 0.7 V. mm. Oxygen tension	
						Mean		Mean
		Göte P.						
1	3.2	Pleurisy	3.8	91.5	652	665	377	380
			4.4	95.2	678		382	
	4.2		3.9	95.5	690	690	368	365
			4.4	95.5	690		363	
	17.2		5.3	92.7	682	688	510	512
			4.8	94.4	694		514	
		Carola C.						
2	5.2	Cardiac incompensation + pleural transudates	4.6	93.9	678	680	363	363
			4.7	94.4	682		363	
	25.2		5.4	91.4	655	671	Hemolysis	
			5.3	95.7	686			
		Erik K.						
3	3.2	Pleurisy	5.2	94.5	673	670	590	592
			5.3	93.7	667		593	
			5.3	94.7	665	664	571	575
			5.2	94.2	662		579	
		Kerstin A.						
4	4.2	Empyema + pneumothorax	4.0	95.8	690	688	511	507
			4.0	94.7	685		503	
		Göte N.						
5	6.2	Pneumothorax	3.7	94.6	681	682	393	391
			4.8	94.8	682		388	
		Ingrid H.						
6	13.3	Pneumothorax	3.5	93.6	673	685	320	320
			3.5	96.7	696		320	
		Eva K.						
7	17.3	Pneumothorax	3.8	96.2	692	693	571	573
			3.5	96.5	693		574	
	8.4		3.5	91.7	645	640	338	336
			3.7	90.3	635		333	
		Karl S.						
8	16.3	Bronchial carcinoma . .	3.3	94.5	678	685	602	599
			3.7	96.8	691		596	

X.

cases.

Oxygen tension difference mm.	Arterial blood					
	Oxygen deficit ml. O_2 pr ml. blood	Oxygen capacity Vol. %	Oxygen deficit % unsaturation	Polarograph − 1.6 V. mm. Oxygen tension	Evacuation method: Oxygen content ml. O_2 pr ml. plasma	Evacuation method: mm. Oxygen tension ($\alpha = 0.0214$)
285	0.0086	16.2	5.8			
325	0.0098	15.9	6.2			
176	0.0053	15.3	3.5			
317	0.0096	19.3	5.0			
43	0.0013		0.7	625 631 } 628	0.0169 0.0164	610 591
78	0.0024	16.4	1.5			
89	0.0027	15.8	1.7	578 569	0.0154 0.0159	555 572
181	0.0055	11.1	5.0			
291	0.0088	16.4	5.4			
365	0.0111	16.0	6.9	332 330		
120	0.0036	14.7	2.5	572 574		
304	0.0092		6.3	345 346		
86	0.0026	15.8	1.6	604 600		

Table

Case	Date	Name Diagnosis	Alveolar air				Arterial blood	
			% CO_2	% O_2	mm. Oxygen tension		Polarograph − 0.7 V. mm. Oxygen tension	
						Mean		Mean
9	16.3	*Ingrid E.* Tuberculous lymph-adenoma	4.0 3.8	93.2 96.2	668 689	679	Hemolysis	
10	5.2	*Viola K.* Pulmonary tuberculosis	3.6 4.0	95.5 94.8	680 675	678	467 473	470
	23.2		4.2 3.8	93.8 96.1	656 676	666	541 536	539
11	1.4	*Sven H.* Postoperative atelectasis	4.4 4.4	95.6 95.2	670 668	679	303 295	299
	2.4		4.6	94.8	663		454 460	457
	4.4		4.8	94.8	666		627 637	632
12	5.2	*Erik N.* Thoracoplasty	4.7 4.4	93.8 94.6	674 683	679	557 560	559
	24.2		5.3 5.0	90.0 93.5	637 662	650	560 562	561
13	17.2	*Helge H.* Thoracoplasty	4.6 4.8	93.8 94.3	690 694	692	628 631	630
14	24.2	*Selma F.* Thoracoplasty	4.8 4.4	94.8 94.9	671 672	672	579 585	582
15	25.2	*Vera K.* Thoracoplasty	4.2 4.1	94.2 94.2	664 664	664	551 546	549
16	12.2	*Anna V.* Cardiac incompensation	3.6 3.3	95.4 96.7	671 680	676	421 426	424
17	4.3	*Karl J.* Cardiac incompensation	3.0 2.5	96.2 96.2	697 697	697	635 632	634
18	4.3	*Arvid H.* Emphysema	3.7 3.1	91.3 96.5	662 698	680	596 591	594

X (continued).

Oxygen tension difference mm.	Arterial blood					
	Oxygen deficit ml. O_2 pr ml. blood	Oxygen capacity Vol. %	Oxygen deficit % unsaturation	Polarograph − 1.6 V. mm. Oxygen tension	Evacuation method: Oxygen content ml. O_2 pr ml. plasma	Evacuation method: mm. Oxygen tension ($\alpha = 0.0214$)
54	0.0016	15.8	1.1	630, 620 } 625		
208	0.0063	14.5	4.8			
127	0.0038		2.6	546 536	0.0145 0.0145	522 522
380	0.0115	17.5	6.6			
206	0.0065		3.5	456 454		
34	0.0010		0.6	627 627		
120	0.0036	18.8	2.0			
89	0.0027		1.5	541 542	0.0154	555
62	0.0019	15.8	1.2			
90	0.0027	11.7	2.3	583 587	0.0166 0.0165	598 595
115	0.0035	13.0	2.7	557 554	0.0150 0.0133	540 (478)
252	0.0076	18.6	4.1			
63	0.0019	20.8	0.9	627 624	0.0172 0.0172	620 620
86	0.0026	19.4	1.3	593 586	0.0151 0.0157	543 566

21. 2. A second determination showed a deficit of 89 mm. corresponding to 1.5 % unsaturation.

Case 13. Helge H. 31 years. *Pulmonary Tuberculosis + Thoracoplasty.* Pulmonary tuberculosis was diagnosed 1935. From 1935 to 1939 pneumothorax. Roentgen examination 24. 1. 42. showed a rarefaction in the right apical region. 29. 1. 42. Thoracoplasty with resection of the first three ribs on the right side. Roentgen examination 5. 2. showed satisfactory collapse of the upper part of the lung, the cavity was pressed medially and downwards.

Afebrile. Sedimentation rate 19 mm./hr.

17. 2. 42. Arterial oxygen deficit during oxygen inhalation 62 mm., corresponding to 1.2 % unsaturation (patient out of bed part of the day sitting in deck-chair during test).

Case 14. Selma F. 39 years. *Pulmonary Tuberculosis + Thoracoplasty.* Tuberculosis of the lungs since 1932. Roentgen examination on 22. 11. 41. In the left lung at the level of the clavicle a large cavity. On 5. 12. 41 and 19. 1. 42 thoracoplasty with resection of seven ribs. Roentgen examination 13. 2. 42 showed a satisfactory collapse of the upper two thirds of the left side of the chest. The cavity could not be clearly distinguished with certainty.

Afebrile. Sedimentation rate 52 mm./hr.

24. 2. Arterial oxygen deficit during inhalation of oxygen 90 mm. corresponding to 2.3 % unsaturation.

Case 15. Vera K. 23 years. *Pulmonary Tuberculosis + Thoracoplasty.* Tuberculosis was diagnosed 1938. Roentgen examination 22. 11. 41. Cavity in the middle part of the left lung. 1. 12. 41 and 21. 1. 42 thoracoplasty with resection of the upper six ribs. Roentgen examination on 6. 2. showed a satisfactory collapse of the upper two thirds of the left side of the chest. The cavity is displaced medially and has diminished to one half.

Temperature slightly above normal. Sedimentation rate 19 mm./hr.

25. 2. 42. Arterial oxygen deficit during oxygen inhalation 115 mm., corresponding to 2.7 % unsaturation.

These four cases are in accordance with each other and show only slight but nevertheless definite pathological oxygen deficit under oxygen breathing. In 3 of the cases the plasty is only about one month old and from the fairly insignificant deficit there may be assumed to be an absence of any considerable formation of atelectasis as a result of the operation.

The statements made in literature concerning the oxygen content of the arterial blood after thoracoplasty are somewhat contradictory. Certain investigators find normal saturation (DECKER 1937, MICHAUD 1938, LAMBERT et al. 1938), while others consider it to be moderately decreased (Mc INTOSH 1935, KALTREIDER et al.

1938). That a possible atelectasis might have been the cause of the decrease in the oxygen deficit does not seem to have been discussed. As will be seen from the cases here described, only a small percentage of the deficit in saturation, however, can be explained in this manner.

Experiments on Animals.

In order to test the possibility by means of the method in question of being able to follow the occlusion of parts of the lung from

Table XI.

Experiment 1. 8. 10. 41. Pneumothorax dxt: Cat.

Time	mm. oxygen tension			Vol. % oxygen content		Vol. % oxygen deficit		% Bloodflow through non-ventilated parts of the lung	
	Alveolar air	Arterial blood[1]	Difference	Arterialized blood[1]	Venous blood	Arterial	Venous		
13^{05}	644	591	53			0.16		2.2	
13^{20}	655	597	58			0.18		2.4	
13^{45}	615	561	54			0.16		2.2	
	(95 % O_2 + 5 % CO_2 in inspired air)								
13^{55}									100 ml. air insufflated into right pleura
14^{00}	656	593	63			0.19		2.6	
14^{18}									60 ml. insufflated
14^{21}	641	526	115			0.35		4.7	
14^{29}									40 ml. insufflated
14^{34}	635	365	270			0.82		11.1	
15^{07}	632	353	279			0.84		11.4	
15^{30}									180 ml. exsufflated from right pleura
15^{33}	665	518	147			0.44		6.0	
15^{43}	665	561	104	19.1	11.7	0.31	7.4	4.2	

Autopsy: Atelectasis around the hilum of the right lung. No atelectasis in left lung.

[1] Arterialized blood = blood that has passed ventilating alveoli i. e. is in tension equilibrium with the alveolar air.
Arterial blood = mixed blood from ventilating and non-ventilating parts of the lung.

ventilation during the course of a pneumothorax, some experiments have been carried out on animals. Pure oxygen has been inhaled through a MÜLLER valve. Alveolar tests have been taken in repeated fractions from the end of the expiration through a tube in the trachea. The arterial sample has been obtained from a cannula in the femoral artery. A sample of the venous blood has been taken either by means of a cannula inserted into the right auricle through the jugular vein or else by puncturing the right ventricle of the heart.

Table XII.

Experiment 2. 17. 10. 41. Pneumothorax sin: Cat.

Time	mm. oxygen tension			Vol. % oxygen content		Vol. % oxygen deficit		% Blood-flow through non-ventilated parts of the lung	
	Alveolar air	Arterial blood	Difference	Arterialized blood	Venous blood	Arterial	Venous		
13^{45}	648	606	42			0.13	5.0*	2.6	
14^{10}	658	633	25			0.08		1.6	
14^{23}									100 ml. air insufflated into left pleura
14^{35}	657	564	93			0.28		5.6	
14^{49}									40 ml. insufflated
14^{55}	658	434	224			0.68		13.6	
15^{30}	656	455	201			0.61		12.2	
15^{45}									20 ml. insufflated
15^{53}	676	413	263			0.80		16.0	
16^{20}									Exsufflation 40 ml. from right, 20 ml. from left pleura
16^{28}	668	500	168			0.51		10.2	
16^{40}	666	538	128			0.39		7.8	
17^{00}									Inhalation of water from the Müller valve
17^{15}	667	226	441	14.6	4.8	1.33	9.8	13.6	

Autopsy: Extended atelectasis of irregular distribution. By comparing the specific weight of the whole lung, air-containing parts and atelectatic parts, these latter are estimated to be 60 % of the total parenchyma.

* The arterio-venous oxygen difference is calculated to be 1/2 of the total capacity.

Table XIII.

Experiment 3. 12. 3. 42. Pneumothorax: Rabbit 3,000 g.

Time	mm. oxygen tension			Vol. % oxygen content			Vol. % oxygen deficit		% Blood-flow through non-ventilated parts of the lung	
	Alveolar air	Arterial blood	Difference	Arterialized blood	Arterial blood	Venous blood	Arterial	Venous		
10^{15}	672	645	27				0.08		1.2	
10^{45}				18.5		11.7		6.8		
10^{55}										100 ml. air insufflated into right pleura.
11^{15}	641			18.7	14.8	5.8	3.9	12.9	30.2	
12^{50}	672			17.1	13.9	5.5	3.2	11.6	27.6	

Autopsy: Right lung completely atelectatic. Atelectasis around the hilum of the left lung.

Experiments 1 and 2 show how the extension of the atelectatic parts gradually increases after establishing a pneumothorax. At first the increase is slow, but once a certain limit of the amount of insufflated air is passed, the atelectasis formation proceeds more rapidly. Experiment 4 shows that when air is insufflated only once the atelectasis does not develop immediately but takes several hours. Experiment 5 seems to point to the same thing.

The successive atelectasis formation made probable by the experiments above described is in agreement with those carried out by HANSON and SJÖSTRAND (1935), in which the atelectasis was macroscopically determined on rabbits which were killed at different intervals after establishing a pneumothorax. It does not seem possible, however, to draw any definite conclusions from their investigations as to when the occlusion of the subsequently atelectatic areas took place, instead it was the moment when all the air had been absorbed that was determined. This absorption takes several hours and can partially explain the approximate 6 hours that HANSON and SJÖSTRAND found for the completion of the atelectasis formation. It seems clear from the above mentioned experiments, however, that this absorption does not entirely explain its successive progress. In these experiments the point of time for the occlusion from the ventilation is shown and not the moment for the absorption of the air.

Table XIV.

Experiment 4. 13. 3. 42. Pneumothorax: Rabbit 2,100 g.

Time	mm. oxygen tension: Alveolar air	mm. oxygen tension: Arterial blood	mm. oxygen tension: Difference	Vol. % oxygen content: Arterialized blood	Vol. % oxygen content: Venous blood	Vol. % oxygen deficit: Arterial	Vol. % oxygen deficit: Venous	% Blood-flow through non-ventilated parts of the lung	
10^{15}	698	596	102			0.31		4.1	
10^{25}									40 ml. air insufflated into right pleura.
10^{35}	680	450	230			0.70		9.2	
11^{00}	690	362	328	17.4	9.8	0.99	7.6	13.0	
12^{25}	687	177	510			1.54		17.3	
12^{40}				16.4	7.5		8.9		
14^{00}	678	229	449			1.36		22.6	
14^{15}				15.7	9.7		6.0		

Autopsy: Extensive atelectasis radiating from the hilum of the right lung. No atelectasis in the left lung.

Table XV.

Experiment 5. 27. 10. 41. Open pneumothorax: Rabbit.

Time	mm. oxygen tension: Alveolar air	mm. oxygen tension: Arterial blood	mm. oxygen tension: Difference	Vol. % oxygen content: Arterialized blood	Vol. % oxygen content: Venous blood	Vol. % oxygen deficit: Arterial	Vol. % oxygen deficit: Venous	% Blood-flow through non-ventilated parts of the lung	
10^{55}	622	545	77			0.28		3.0	
11^{10}	644	582	62			0.19		2.6	
12^{05}	674	585	89	15.8	8.1	0.27	7.7	3.5	
12^{17}									Open pneumothorax dxt. Diameter of the opening 2,5 mm.
13^{15}	641	342	299			0.91		10.6	
13^{25}				14.1	5.5		8.6		
13^{30}	641	<150	>500			>1.5		>17.5	
14^{10}				13.3	10.8	2.5		29.1	

Autopsy: Right lung almost completely atelectatic. Rather extensive atelectasis around the hilum of the left lung (about 1/3 of the parenchyma of this lung).

Table XVI.

Experiment 6. 30. 1. 42. Double pneumothorax: Rabbit.

Time	mm. oxygen tension			Vol. % oxygen content			Vol. % oxygen deficit		% Blood-flow through non-ventilated parts of the lung
	Alveolar air	Arterial blood	Difference	Arterialized blood	Arterial blood	Venous blood	Arterial	Venous	
11^{20}	648			19.3	16.3		3.0		36.6
12^{05}	677	113	562				1.70		20.7
13^{20}	672	322	350	16.0		7.8	1.06	8.2	12.9
14^{20}	668	360	308				0.98		11.2
14^{50}				16.0		7.7		8.3	
15^{40}	673	373	300				0.91		11.0

During the attempt to expose the right ventricle of the heart before the experiment, the pleurae on both sides were lesioned. The openings were immediately shut and air exsufflated.

Autopsy: Rather extensive atelectasis radiating from the hilum in both lungs.

In the cases in question, moreover, these two points of time all but coincide with each other, owing to the fact that the animals breathe pure oxygen, which is absorbed much more rapidly than the nitrogen which is enclosed in the alveoli when ordinary air is breathed (LICHTHEIM 1879).

The effect of exsufflation of part of the injected quantity of air will be seen from the latter part of experiments 1 and 2. The same thing is also illustrated by experiment 6. The regression of the atelectasis is at first rapid, but later it seems to proceed slowly. The conditions for the re-inflation of an established atelectasis are clearly not so favourable as is the case with the foetal atelectasis, where a complete inflation with air seems possible by only some few breaths. When making roentgenological studies of the regression of an experimental obturation atelectasis on dog, HELLER (1914) found that the greater part of the reinflation could take place with a few breaths, when once begun. Before starting, however, it could take any time from a few minutes to a couple of hours, all according to the age of the atelectasis. The parts round the hilum, where the first signs of atelectasis were discovered were re-inflated last. Generally speaking, however, the regression of the atelectasis was a far more irregular process than the formation. The conditions for a complete regression of the atelectases in the

above mentioned tests are naturally not so satisfactory as they were for HELLER, it being impossible to remove all insufflated air. This will explain why the arterial oxygen deficit did not reach the original values. The autopsy after the experiments showed a remaining atelectasis around the hilum.

Discussion.

As soon as an atelectasis has developed, the majority of experiments point to a decrease in the blood-flow. From MOORE's (1931) experiments the size of this decrease may be estimated at c. 50 %. That there is a continued decrease in this blood-flow with the age of the atelectasis is very probable, owing to the atrophy going on in the atelectatic parts. This atrophy has been established experimentally by weighing atelectatic lungs after a pneumothorax of long standing (HANSON and SJÖSTRAND 1935). When examining human lungs it is difficult to judge the additional effect of simultaneously existing infection and the formation of the connective tissues (LINDBLOM 1921). If there is no infection in an atelectatic part there is only a slight increase of the connective tissue (TOMASCEWSKI 1917, LOESCHKE 1928, REICHEL 1933, ADAMS et al. 1935) and the lung retains its structure thus rendering possible a resumption of the function on the suspension of the collapse.

Few direct attempts have been made to determine the relation of the blood-flow with the age. One isolated experiment made by CORPER and RENSCH (1920), where they observed a decreased colouring of the lung by a scarlet-red injection after 4 weeks' pneumothorax does not throw any definite light on the question. According to SACKUR's principle ADAMS, HRDINA and DOSTAL (1935) found that the blood-flow through the atelectatic lung did not show any definite decrease even though the bronchial occlusion had lasted as long as 70 days. The determinations were carried out under artificial breathing with insufflation of air in the trachea, and the positive pressure thus arising in the alveoli of the ventilated lung must have rendered the blood-flow through this lung more difficult and increased the relative blood-flow of the atelectatic lung.

That an atelectasis of long standing and not complicated by infection can really cause a strong decrease in the blood-flow is best illustrated in case 3, where there was a total collapse of one lung through pneumothorax and exudation, and which had been going

on for several months. Cases 8 and 9 with atelectases caused by bronchial stenosis point to the same thing. In case 10 the decrease in blood-flow could be ascertained by comparing two determinations made with an interval of three weeks. That the age of the process influences the existence of an arterial oxygen deficit was pointed out already by HÜRTER in connection with an acute pleurisy with a definite pathological deficit, and a complete compression of the lung due to a pneumothorax of long standing with exudation, showing a normal saturation. Besides age the presence of an infection (see cases 9, 10 and 11) or a positive pressure in the pleural cavity (cases 3 and 7) may also contribute to a decrease of the blood-flow.

That this strong decrease in the blood-flow is not, however, a process developing already during the first few hours or days after the atelectasis has begun is obvious from several cases (see cases 1, 2, 5 and 6). It should therefore be possible to follow the development of atelectases with the method described, e. g. during the establishment of a pneumothorax. After leaving the original compression principle when treating tuberculosis and instead aiming at a selective pneumothorax with the preservation of the function of the healthy areas (ASCOLI 1930, BARLOW and KRAMER 1922), it may at least be of theoretical interest to see how far this aim to avoid an extended atelectasis formation has proved successful. The present method seems in this respect to be able to complete the roentgenological diagnosis, which presumes a localized atelectasis of none too small an extent and is not possible for small multiple atelectases, e. g. the parenchymatous form observed in thoracoscopy by JACOBÆUS (1934) or the fan-shaped atelectases in the region of the hilum, which, at least, in laboratory animals represent the first stages of the formation of atelectases in pneumothorax (HANSON and SJÖSTRAND 1935).

The oxygen deficit in the arterial blood which is established in every case with collapse of the lung is naturally not only to be found in the present experimental conditions, but amounts to the same value when inhaling ordinary air. The size of this deficit is only in a small number of cases, however, sufficient to be able to be proved by the usual determination of the arterial oxygen saturation according to VAN SLYKE, and this explains why the saturation in many cases is not considered to be affected by the collapse therapy.

If 92 % is taken to be definitely the lower limit for the normal

saturation. 5 % (97—92 %) lies within the limits that can be due to e. g. a different affinity between hemoglobin and oxygen, but can just as well be an expression for an admixture of venous blood. In order to bring about this reduction of 5 %, it is necessary for about 15 % of the total blood-flow to pass through non-ventilated parts. If the blood-flow is reduced to half through an atelectatic area, 30 % of the total pulmonary parenchyma might thus be atelectatic without causing any decrease in the arterial oxygen saturation detectable in an ordinary determination of the oxygen content.

Apart from the blood-flow through non-ventilated parts an arterial oxygen deficit may also arise from an imperfect renewal of the alveolar air resulting from the changed conditions of ventilations that the presence of an exudate or a pneumothorax may cause. This impaired ventilation is dependent inter alia on the stability of the mediastinum and the existence of indurated parts which affect the mobility of the surrounding pulmonary tissue. As the co-operation of these factors must be extremely different in each particular case, it is easy to explain why no unanimous conception has been formed as to whether collapse therapy causes an oxygen deficit or not (Hürter 1912, Le Blanc 1922, Lundsgaard and Möller 1922, Hilton 1925, Meakins and Davies 1925, Richard et al. 1932, Ebina 1932, Lorenz and Wüllenweber 1932, Mc Intosh 1935, Christie and Mc Intosh 1936, Decker 1937, Michaud 1938, Kaltreider et al. 1938, Lambert et al. 1938). That already that part of the oxygen deficit, which is due to the existence of atelectasis, is subject to great variations in each case is illustrated by a comparison between the above-mentioned cases. It will be seen that the age of the atelectasis as well as its size is decisive for the deficit.

The Oxygen Content of Arterial Blood when breathing Air and inhaling Oxygen.

In connection with the determination of the oxygen deficit in the arterial blood during oxygen inhalation, the oxygen content of the arterial blood has been determined in some cases even while breathing ordinary air. These are intended to show how it is possible to analyse an existing oxygen deficit by determining one of the factors which bring about a deficiency of oxygen saturation

in the arterial blood, in this case the admixture of venous blood from non-ventilated parts of the lung.

Case 16. Anna V. 51 years. *Cardiac Incompensation.* Valvular disease was diagnosed 1910. Since 1937 increasing dyspnoe, since 1939 swollen legs and cyanosis. On admission to hospital on 22. 1. 42 the patient showed pronounced incompensation, the veins of the neck were distended, the edge of the liver was palpated c. 5 cm. below the costal margin, dulness on percussion of the flanks, œdem over the sacral region, abundant moist râles over the lungs. Circulation time (decholin test) beginning 34 sec. max. 70 sec. end 80 sec. Venous pressure above 32 cm.

12. 2. 42. Slowly recovering, no dyspnoe at rest. Still cyanosis and swollen legs.

Arterial oxygen deficit during oxygen inhalation 252 mm., corresponding to 4.1 % unsaturation.

13. 2. Arterial oxygen content while breathing air 16.6, 16.6 vol.%, oxygen capacity 20.1, 20.2 vol.%. Oxygen saturation 82.6 %.

Compared with a normal saturation value of 95 there is in this case an arterial deficit of 12.4 % unsaturation. As the deficit when inhaling oxygen is 4.1 only 1/3 of the normally existing deficit can be explained by admixture of venous blood.

Case 17. Karl J. 59 years. *Cardiosclerosis + Cardiac Incompensation.* Heart trouble since 1932 with pains in the chest, palpitations, dyspnoe cough, œdem of the ankles. Occasional cardiac asthma.

4. 3. 42. Pronounced dyspnoe even at rest, some cyanosis, oxygen gave him good relief.

Arterial oxygen deficit during oxygen inhalation 63 mm., corresponding to 0.9 % unsaturation.

Arterial oxygen content while breathing air 13.9, 13.9 vol.%. Oxygen capacity 20.3, 20.4 vol.%. Arterial oxygen saturation 68.4 %.

The patient died a few days later. Autopsy showed chronic stasis of most organs, no pneumonia but congestive induration and hæmorrhages of the lung.

This case showed a pronounced deficit amounting to 95 — 68.4 = 26.6 % unsaturation. This deficit practically disappears completely when pure oxygen is inhaled, and only 0.9 % can be attributed to an admixture of venous blood.

Case 1. (see p. 60).

The oxygen deficit in the arterial blood when breathing ordinary air was 95 — 90.5 = 4.5 % unsaturation. When inhaling oxygen the deficit was 5.3 and 6.2 %, and explained in this case the entire difference. There was no other cause for the oxygen deficit in the arterial blood while ordinary air is being inhaled.

Case 18. Arvid H. 44 years. *Pulmonary Emphysema.* For 4 years distressing dyspnoe, especially during the winter and when out of doors. Slight cough in the morning. Roentgen examination 28. 2. 42. Nothing pathological from the lungs except some dense strips in fourth and fifth right interstitium, against which some distended bronchi stand out.

Afebrile. Sedimentation rate. 2 mm./hr.

4. 3. 42. Arterial oxygen deficit during oxygen inhalation 86 mm., corresponding to 1.3 % unsaturation (patient out of bed most of the day, sitting in deck-chair during test).

Arterial oxygen content while breathing air 18.3 vol.%. Oxygen capacity 19.5, 19.3 vol.%. Arterial oxygen saturation 94.4 %.

In this case saturation is practically speaking normal. When determining the oxygen deficit while oxygen is being inhaled, a small but none the less definite pathological value of 1.3 saturation % is obtained. The case illustrates how behind a normal value of oxygen saturation a definite pathological deficit can be concealed, owing to the difficulty of stating the normal value exactly. The deficit can be proved, however, when oxygen is inhaled, owing to the uniform composition of the alveolar air and the complete tension equilibrium between it and the blood, by means of which the normal value can be calculated far more exactly.

Case 19. Uno E. 27 years. *Morbus coeruleus* (FALLOT's *tetralogy?*). Valvular disease from birth, the patient has never been able to move about like boys of his own age, has always had blue lips and cheeks. Dyspnoe has been increasing for $1^1/_2$ years.

Status: No dyspnoe at rest, very pronounced cyanosis, clubbing of fingers and toes. Heart: slight voussure, systolic thrill, harsh systolic murmur with maximum over left sterno-clavicular joint. P 2 accentuated. Roentgen examination: The size of the heart 630 cc. pr sq.m. body surface. Configuration normal, no bulging of pulmonary artery, no congestion of the lung. Transposition of aorta.

4. 3. 42. Arterial oxygen content while breathing air 19.0, 19.05 vol. %, oxygen capacity 31.8 vol.%. Arterial oxygen saturation 59.8 %. Arterial oxygen deficit (the normal saturation is assumed to be 95 %) $31.8 \times 0.95 - 19.0 = 11.1$ vol.%.

Arterial oxygen content during oxygen inhalation 24.3, 24.4 vol.%. Calculated value for the oxygen content of blood in lung veins 31.8—0.7 (physically dissolved oxygen when saturating blood with air at room temperature) + 2.1 (physically dissolved oxygen at an alveolar oxygen pressure of 692 mm) = 33.2 vol.%. Oxygen deficit of mixed arterial blood $33.2 - 24.35 = 8.85$ vol.%.

Owing to congenital heart disease, this case showed a maximum admixture of venous blood, which does not, however, come from non-ventilated parts of the lung, but goes straight from the right

to the left side of the heart most likely through a septum defect. The surplus of physically dissolved oxygen existing when oxygen is inhaled is here insufficient to saturate the hemoglobin in the admixed venous blood, but a considerable amount of non-oxygenated hemoglobin is to be found in the arterial blood even during oxygen inhalation. The oxygen deficit, as will be seen from the calculations, is almost as great when breathing ordinary air as when inhaling oxygen, and can thus be solely or largely attributed to the admixture of venous blood from the right part of the heart to the fully oxygenated blood coming from the lungs.

A comparison between cases 17 and 19 demonstrates in a striking manner the different effects of oxygen therapy on two separate types of impaired arterial oxygen saturation. The one case probably concerns a strongly reduced diffusion capacity through the pulmonary epithelium. When there is a high tension difference between the alveolar air and the blood, the reduced diffusion constant will allow a complete saturation of the hemoglobin with oxygen. In the other it is probable that the blood passing the lung becomes normally saturated with oxygen, and oxygen breathing cannot affect the oxygen content of the mixed arterial blood, except by increasing saturation from 95 to 100 % of the blood passing the lung and by the presence of an increased fraction of physically dissolved oxygen in this blood.

In the case of oxygen inhalation, as it is usually carried out in the clinics, i. e. with the use of nasal catheters or open masks, it is not possible to attain a higher oxygen content in the alveoli than c. 40 %. In such cases where an arterial oxygen deficit is due to diffusion difficulties, this concentration is no doubt satisfactory, owing to the extra tension difference of 180 mm. that it creates between the alveoli and the blood; the same will be the case when the deficit is due to an unsatisfactory ventilation of certain parts of the lung.

In order to be able to achieve something with oxygen therapy, where the oxygen deficit is due to an admixture of venous blood, attempts must be made to increase the physically dissolved oxygen as much as possible, i. e. to raise the oxygen content of the alveolar air to such a degree as is possible, bearing in mind the sensitivity to high oxygen concentrations of the alveolar epithelium.

Conclusions.

The oxygen deficit in the arterial blood during oxygen inhalation is a quantitative expression for the admixture of venous blood from non-ventilated parts of the lungs. Oxygen inhalation practically speaking eliminates an inequality in the composition of the alveolar air and the arterial deficit due to an impaired diffusion through the epithelium of the lung.

There is a surplus of physically dissolved oxygen in arterialized blood during oxygen inhalation, which is used in the first place to saturate the hemoglobin in the venous blood coming from non-ventilated parts of the lung. By eliminating the chemically combined oxygen through centrifuging, the physically dissolved oxygen alone can be determined, thus ensuring an increased accuracy. In consequence of the direct connection between the oxygen tension and the quantity of physically dissolved oxygen, the determination of the latter implies a simple method for the determination of the oxygen tension.

As all hemoglobin appears in oxygenated form, the blood-cells can be separated from plasma at a low temperature, thus avoiding the oxygen consumption existing at the temperature of the body in blood or plasma. Nothing has been able to prove the existence of an adsorption process by which the oxygen content of the plasma could be decreased by centrifuging.

When testing plasma, the polarographic current registered at — 0.7 V. and — 1.6 V. (as compared with a saturated calomel electrode) is a specific expression for the oxygen tension and content. The current is increased when making polarographic measurements on blood owing to the presence of chemically combined oxygen. The size of this increase is an expression for the dissociation velocity of the oxy-hemoglobin and the diffusion rapidity through the blood-cell membrane.

The error of the polarographic method amounts to 1 % of the value registered. It is superior to the evacuation method both as regards accuracy and rapidity.

The error of the evacuation method is c. 0.08 c.mm., and allows of a determination of the physically dissolved oxygen in 0.2 ml. plasma. In order to make an analysis of the gases in blood with the same apparatus 10 c. mm. blood is required.

Normal subjects showed an arterial deficit amounting to 11.3 $\pm$ 1.5 mm. when inhaling oxygen, which means that 0.6 % of the total blood-flow passes by ventilated alveoli.

Patients with healthy lungs confined to their beds showed in some cases an increased deficit. This is attributed to a formation of atelectasis due to lying in bed.

A number of determinations have been carried out on patients with atelectases caused by compression or obturation. The blood-flow through an atelectatic area may be estimated at c. 50 % of the normal during the first few days, but will subsequently diminish, in some cases considerably.

The formation of atelectasis after the establishment of a pneumothorax has been followed in experiments on rabbit and cat by means of repeated determinations of the arterial oxygen deficit. It is maintained that there are possibilities of similarly following the formation of atelectasis when treating tuberculosis with artificial pneumothorax.

In some cases of heart and lung diseases the arterial oxygen deficit has been determined when breathing air as well as during oxygen inhalation. In this manner it has been possible to calculate that fraction of the oxygen deficit which is due to an admixture of venous blood from non-ventilated parts of the lung or from defects in the wall between the right and left side of the heart.

Summary.

In order to be able to analyse more closely the arterial oxygen deficit existing under many different pathological conditions, it is desirable to isolate the different factors, which influence the arterial oxygen saturation (the composition of alveolar air, the diffusion through the alveolar epithelium, the admixture of venous blood to arterialized blood). In the preceding chapters a method has been described which renders it possible to determine that part of the deficit due to the blood-flow through non-ventilated parts of the lung. Its principle is that during oxygen inhalation the oxygen tension difference between alveolar air and arterial blood is due only to an admixture of venous blood. By inhaling pure oxygen and thus eliminating all nitrogen, a uniform composition of the alveolar air is obtained, and even within insufficiently ventilated areas of the lung, the oxygen content will be high enough to bring about a complete oxygen saturation of the hemoglobin. With this high oxygen tension there will also be an all but complete oxygen tension equilibrium between alveolar air and blood, even when diffusion is impaired. The oxygen tension of the alveolar air can therefore be used as a measure of the oxygen tension of blood passing ventilated alveoli. The oxygen tension of the arterial blood has been determined by making use of the direct proportionality between the tension and the amount of physically dissolved oxygen. The chemically combined oxygen is eliminated by centrifuging the blood and the analyses are then made on plasma where the oxygen occurs only in the physically dissolved form.

Two methods have been used for the determination of physically dissolved oxygen.

1. The evacuation method, which is a further development of the manometric principle for determining gases extracted in vacuum, introduced by VAN SLYKE and NEILL. Instead of the 5—10 ml. plasma necessary for a determination in their usual apparatus only 0.2 ml. will be needed. The error for a single determination will be 0.04 volume % or 0.08 c.mm.

2. The polarographic method according to HEYROVSKÝ and VÍTEK. At the surface of a mercury drop electrode oxygen will be reduced at a certain potential and create a current, the strength of

which is directly proportional to the oxygen content. The error of this method is half that of the evacuation method or 1 % of the value obtained.

The evacuation method has chiefly been used to control certain data of the polarographic method. In consequence of the rapidity with which the analyses can be performed, the polarographic method is the one almost solely used in the experimental and clinical determinations.

Measurements with the polarographic method have also been carried out in the presence of chemically combined oxygen. This causes an increase of the current, which depends on the dissociation velocity of the oxyhemoglobin. The variations found are in agreement with investigations made by HARTRIDGE and ROUGHTON on the kinetics of hemoglobin.

The elimination of the chemically combined oxygen by centrifuging the blood can take place at 0°, when there exists a surplus of physically dissolved oxygen, since the increased affinity between oxygen and hemoglobin, which is caused by lowering the temperature, cannot assert itself unless there is hemoglobin in unoxygenated form. By centrifuging at low temperature the consumption of oxygen by the blood need not be taken into consideration. With the above-mentioned method for determining the oxygen deficit of arterial blood caused by non-ventilating parts of the lung, there will be a similar surplus of physically dissolved oxygen owing to the inhalation of pure oxygen. When some parts of the lung do not ventilate, this surplus will be diminished, but under most pathological conditions it will still be sufficient to bring about a complete oxygenation of the hemoglobin in the blood from the non-ventilated areas.

If the oxygen deficit of both venous and arterial blood is known, it is possible to calculate the blood-flow through the non-ventilated areas of the lung, a principle first adopted by SACKUR. The oxygen deficit of venous blood can be obtained from the arterio-venous oxygen difference, as determined e. g. in the GROLLMAN method for the cardiac output. In this work no such determinations, however, are made, but values from the literature on this subject have been used.

The oxygen tension difference between alveolar air and arterial blood during oxygen inhalation in normal subjects is 11 ± 1.5 mm. With a normal arterio-venous oxygen difference this means that 0.6 % of the total blood-flow is short-circuited past the open

alveoli. Anatomically this blood may come from the bronchial veins.

Patients confined to bed but pulmonarily healthy show in some cases a normal difference of oxygen tension, but there is often an increase definitely pathological. This is attributed to a formation of atelectasis, caused by the impaired ventilation of the basal parts of the lung, which comes from lying in bed.

Patients with definite pathological conditions of the lungs often show great differences in the oxygen tension between alveolar air and arterial blood far exceeding those that can be attributed to lying in bed. The investigations have been made chiefly on patients with a collapse of the lung due to bronchial occlusion or compression from pleural exudate and pneumothorax. Distinct connection is found between this difference on the one hand and the size and age of the atelectasis on the other. The oxygen tension differences point to a blood-flow through an atelectatic part, which amounts to about 50 % of the normal during the first days and weeks, but afterwards it decreases considerably in some cases.

As the blood-flow through a recent atelectasis does not seem to be decreased by more than about 50 %, it is possible with the method described to follow the formation of atelectasis, e. g. in the course of a pneumothorax. This has been illustrated by some experiments on rabbit and cat. After establishing a pneumothorax the oxygen tension difference by and by increases as a sign of the successive formation of atelectasis. By means of this method there seems to be a possibility of following the formation of atelectasis in the course of the collapse therapy against tuberculosis. In order to be visible on a Roentgen plate the atelectasis must be of a certain size. Small multiple atelectases, e. g. the parenchymatous form described by JACOBÆUS are not visible on the Roentgen plate.

In a few cases the oxygen deficit of arterial blood existing when breathing ordinary air as well as the deficit that remains during the inhalation of oxygen has been determined. As the deficit during oxygen inhalation is due to an admixture of venous blood from non-ventilated parts of the lung (or to blood which in some other manner is short-circuited past ventilating alveoli), it is in this way possible to calculate that part of the deficit during air-breathing which is to be attributed to a similar short-circuit.

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THE RESPONSE TO LINEARLY INCREASING CURRENTS IN MAMMALIAN MOTOR AND SENSORY NERVES

BY

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MED. LIC.

THESIS,
with the sanction of the Royal Caroline Institute submitted to public defense at the Great Lecture Theatre of the Institute on May 7th, 1942, at 1.00 o'clock.

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CONTENTS.

PREFACE

Work in this laboratory on accommodation and repetitiousness in mammalian nerves had shown that it was necessary to develop a new technique for satisfactory control of form and strength of the electrical stimulus in order to obtain an adequate method of measuring accommodation. Out of this necessity arose the theme of my paper.

It is a pleasure to record my gratitude to the Director of this Laboratory, Professor RAGNAR GRANIT, for constant advice and criticism during the experimental work.

I am also greatly indebted to the physicist of the Neurophysiological Laboratory, Mr T. HELME, for the design of the stimulator and repeated checking of the apparatus.

This work has been supported by a Rockefeller grant to the Neurophysiological Laboratory as well as by personal grants to the author from the foundation "Therese och Johan Anderssons Minne", "C. A. Nobels fond" and the "Lindahls fond" of the Royal Swedish Academy of Sciences.

Stockholm, April 1942.

Carl Rudolf Skoglund.

Introduction and Problem.

The theme of this work is an analysis of the response to linearly increasing currents carried out with a technique enabling simultaneous recording of the form of the stimulating current and the ensueing response in terms of action potentials in relatively isolated motor or sensory fibres. To this end an apparatus was built (see below, section on Technique) delivering linearly increasing currents of strictly controllable gradient and strength through the anode circuit of a valve connected to shift the one beam of a double-cathode ray in proportion to the current intensity stimulating the nerve, while at the same time the impulses in the nerve are recorded through an amplifier coupled to the other beam. It is a well known fact (see Historical section) that a decreased rate of rise of the stimulating current then has to be compensated for by increased current strength. How this fact turns up in my experiments is shown by fig. 8 *b* and *c* in which two currents rising at different rates are seen to elicit a response which is delayed by the slower current by a certain amount. In the latter case it has also been necessary to use a stronger stimulus in order to excite.

Hitherto, in work of this character, a threshold muscle contraction has been used as index of an excitatory effect. When records have been taken from the nerve, the whole nerve has been put on the recording electrodes as a general check on rhythmicity of the discharge. The rate of rise of the current has not been simultaneously recorded and hence it has been impossible both to know for each gradient *what* happens and *when* it happens, in terms of exact current strength at the time when the discharge begins. It will be shown that the information obtainable by the technique used in this work is of significance for the elucidation of a number of questions regarding "accommodation", electrotonus and "repetitiousness" in different types of mammalian nerves.

In recent times the most important contribution to the problem of "accommodation" has come from Hill and his collaborators

(HILL, 1936, SOLANDT, 1936 a and KATZ, 1936). Neglecting for the moment the full theoretical background of HILL's work it is simply but somewhat schematically described by reference to fig. 1 in which the current necessary for a threshold muscle contraction is plotted in multiples of rheobasic strength against a factor proportional to the rising time of the current. The figure shows these curves to rise linearly but with different slopes. Thus, if the curves rise steeply, a relatively greater increase of current strength has been necessary to compensate for increasing time constants of the stimulus along the abscissa. According to HILL this may be interpreted as a rise in the threshold of the nerve evoked by the stimulating current, a rise which has the further property of continuing exponentially during stimulation. Since now one of the effects of the slowly rising stimulus is to increase the threshold of excitation it is clear that, unless this rise of threshold is compensated for by a stronger stimulus, the latter remains subthreshold. This "rise of threshold" is a concept synonymous with *accommodation.*

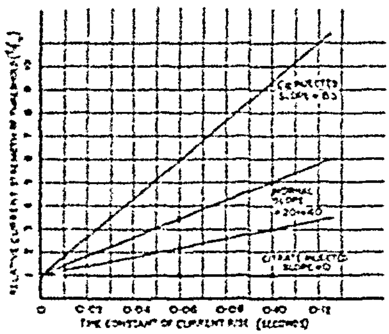


Fig. 1. From SOLANDT (1936 b). See text.

The uppermost curve of fig. 1 has high accommodation, the curves below it, less accommodation. For theoretical reasons HILL uses the inverse value of the slope, his constant λ rather than the slope itself as a measure of accommodation.

The significance of this definition of the concept of accommodation lies in the facts that it is simple and at the same time obviously somehow related to the ease with which nerves can be made to discharge repetitively. The degree of "repetitiousness" may preliminarily be defined as a greater or lesser degree of accommodation to the stimulus, and, actually, it has been shown by SOLANDT (1936 a) and KATZ (1936) that the smaller the slope of the "accommodation" curve of fig. 1, the easier the nerves discharge repetitively. Somehow therefore the slope of the curve is a measure of to what an extent, a nerve "accommodates" to a stimulus or lacks this capacity. An important consequence of HILL's work was the demonstration by SOLANDT (1936 a) that

the increased irritability and repetitiousness following removal of Ca-ions is accompanied by a decrease of the constant λ. The results of SOLANDT with decitrated nerve or nerve bathed in an environment deficient in Ca-ions have been confirmed by SCHRIEVER and CEBULLA (1938). SOLANDT (1936 a) has further shown that the great differences in the degree of repetitiousness, known to exist with respect to stimulation with continuous currents in nerves of different animals (FESSARD, 1936), also can be correlated with variations of λ.

In work of this character the experimenter sooner or later encounters a difficulty inherent in the method by which accommodation curves are being measured. This question will be discussed with more attention to detail in the Historical section. Suffice it here to mention that this difficulty is a direct consequence of the methodical limitations set by the use of a constant muscle contraction as index of the excitatory effect. It was known already by v. KRIES (1884) and HOFFMANN (1910) that slowly rising currents lead to repetitive discharges which greatly modify the size and form of the muscle contraction. HOFFMANN noted that the muscle action currents then indicated repetitive firing, as also later found by SCHRIEVER and CEBULLA (1938). GRANIT and SKOGLUND (1941) made similar observations with mammalian nerves for which, as a rule, the accommodation curves soon rose to a level where they gradually turned round to become horizontal. At the same time the muscle contraction became drawn-out and the nerve was found to discharge repetitively. For this change the authors introduced the convenient term "breakdown of accommodation". It is clear that it also means breakdown of the index of accommodation.

In this dilemma there are two alternatives: either to restrict one's measurements to the early part of the accommodation curve where it follows a straight line giving the constant λ or to work out — unprejudiced by theory — another index for such measurements in terms of what happens in a restricted population of motor or sensory nerves. I have chosen the latter alternative. One of the central themes of this work is an analysis of this question which soon was found to require the technical development briefly alluded to above.

At the same time this work has been motivated by a desire to develop the points of view set forth by BERNHARD, GRANIT and

SKOGLUND (1942) in a paper on breakdown of accommodation in relation to nerve as model sense organ. These authors have suggested as a working hypothesis that the higher sense organs excite their afferent neurones by means of a "generator" potential spreading electrotonically down the axons. From these aspects the question as to how nerve reacts to slowly rising currents becomes of particular interest. The nerve so excited also serves as model sense organ.

In support of this hypothesis BERNHARD, GRANIT and SKOGLUND have in the first instance drawn upon the results with the retina. They point out that "the simpler the retina or the better the isolation of components of the response of complex retinae, the more definite the evidence for the conclusion that the generator mechanism is a slowly rising potential preceding excitation or inhibition as the case may be" (see ADRIAN and MATTHEWS, 1927, HARTLINE and GRAHAM, 1932, HARTLINE, 1938, GRANIT, 1933, 1938, GRANIT and THERMAN, 1935, BERNHARD, 1942 a and b). That this assumption is reasonable is also emphasized by the exciting properties of "local" potentials in nerve (ARVANITAKI, 1936, HODGKIN 1938), muscular end-plates (GÖPFERT and SCHAEFER, 1937, ECCLES, KATZ and KUFFLER, 1941, ECCLES and KUFFLER, 1941), salivary glands (LANGENSKIÖLD, 1941), spinal neurones (UMRATH, 1933, BARRON and MATTHEWS, 1938). From time to time many authors have taken it up in one form or another, among them, for instance, ADRIAN (1932), BARRON and MATTHEWS (1938), ERLANGER and BLAIR (1936) apart from those working on the retina.

If long-lasting discharges are to occur, as a consequence of the proposed hypothesis, in response to a generator potential in a sense organ, the latter must influence a type of nerve in which accommodation on the whole is insignificant. For frog's nerve it has actually been found by ERLANGER and BLAIR (1938) that sensory nerves are more repetitive than motor nerves. From the recent work of v. BRÜCKE, EARLY and FORBES (1941) it is, however, clear that there are differences between mammalian motor and sensory nerves which are not reproduced by bullfrog nerve. For this reason, but also in order to obtain quantitative information from experiments with linearly rising currents imitating generator potentials -- a procedure very different from the one used by ERLANGER and BLAIR — it was deemed important to use a mammalian preparation, which also in many respects is a stabler

and more reliable preparation than the frog. We shall see that there are significant differences in the response of mammalian motor and sensory nerves to linearly rising currents and that these differences are *not* wholly covered by the simplification now known under the term accommodation. The hypothesis inspiring these experiments has given an added interest to these differences.

Historical Section.

1. Slowly Rising Currents.

In the introduction it has already been shown above how HILL's accommodation constant λ can be derived from experimentally determined accommodation curves. Theoretical derivations of the time-constant of accommodation have been made by RASHEVSKY (1933), MONNIER (1934) and HILL (1936). These time-constants essentially express the same fact and stand in a simple mathematical relationship to each other as well as to the experimentally derived constants, as is evident from the papers referred to. A schematic review has also been given by SCHAEFER (1940).

In consideration of the special points of view to be emphasized in this work in connexion with criticism of the experimental methods it is perhaps best to begin by describing the technique of HILL and his associates (SOLANDT, 1936 a). They have used an apparatus of the type introduced by LAPICQUE (1926). In this apparatus, as is well known, currents with an exponential rise are obtained by discharging condensers over variable resistances. The rising times in msec. are given by the product of capacity in μF and the resistance in thousands of ohms. The rheobase is first determined with a threshold muscle contraction as index, then the current strength necessary for the same threshold when the stimuli rise more slowly. In actual practice voltages are being measured. The threshold voltages — proportional to threshold currents — are divided by the rheobase and plotted against the corresponding time-constants of current rise. The result is a straight line the slope of which, as we have seen, is equal to the reciprocal of the time-constant λ of accommodation.

For the sake of comparison could be mentioned some other variants of the use of exponential currents. SCHRIEVER (1931, 1932) stimulates with a current strength twice the rheobase and measures the *Halbwertszeit* of the exponential rise which then still

excites. This rising time he calls *Einschleichzeit*. LAPICQUE (1937 a) determines the slowest exponential rise, which at rheobase strength still is capable of exciting, and calls the time-constant of this rise *seuil de climalyse*.

There have also been a number of attempts to use linearly rising currents (*e. g.* v. KRIES, 1884, LUCAS, 1907, FABRE, 1927).

The method introduced by v. KRIES may be regarded as the prototype of HILL's method. v. KRIES obtained linearly rising currents by the rheonome technique and measured the voltage

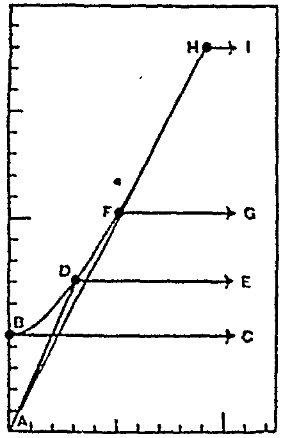


Fig. 2. From LUCAS (1907). Abscissae: duration of current rise to full strength. Ordinates: Current strength required for threshold muscle contraction.

"The point B marks the current strength required to excite when the current was turned on instantaneously by means of a key. D, marks the current strength required when the current reached its full value in 0.031 sec. F, the current strength required when the current reached its full value in 0.051 sec, and so on. In other words, the current-variations required to excite are represented by ABC, ADE, AFG and AHI. It is clear at once that there is a minimum current-gradient, represented by the line AFH and that any gradient less steep than this will fail to provoke a twitch of the muscle, even though the current-strength finally reached be considerably increased."

necessary for a muscle contraction. This value divided by the rheobase was called excitation quotient and could be determined for every rising time. The greater the excitation quotient, the greater the accommodation, this quotient therefore being a factor proportional to the reciprocal of λ.

LUCAS's (1907) technique for producing linearly rising currents is similar in principle. He also presents measurements checking the linearity of the current which was found to be good except in the last phase of the rise. He also measured threshold current strength for different rising times and plotted them against the

latter. Owing to the fact that the current gradients also were drawn, his method of plotting is superior to earlier methods of presenting the results, and, as we shall see, also to later methods. In his experiments on toads and frogs LUCAS came to the conclusion that there was a "minimal current gradient" below which no stimulus excited, even if raised to 8 times the rheobase. (For details, see fig. 2).

FABRE (1931 a) has used the same observation — *pente limite* — for determining his own *constant linéaire* which is the rheobase divided by this threshold current gradient, a time factor which in reality is identical with λ. With both exponentially and linearly rising currents one therefore reaches the same end result with regard to the time constant of accommodation, as is also required by the theoretical treatments of the problem (see HILL, 1936).

After this review of representative work with the different methods it should, however, be emphasized that the linear type of current rise is a more satisfactory instrument in a detailed analysis of the relation between current gradient and excitation. Already LUCAS (1907) pointed out that the exponential type of current, which had then been used by GILDEMEISTER (1904), was unnecessarily complicated since in this case there was no simple relation between current gradient and current strength. Considering the exponential curve as a whole it is clear that in order to obtain a certain prescribed slope for the "stimulating" part of the curve one will be forced to accept an additional amount of slower current rise which can exceed the value necessary for a threshold excitation. Accepting the experimental fact that the current gradient is an essential factor in the elicitation of the excitatory effect it is obvious that the steep part of the exponential current determines the excitatory effect and that, consequently, the later gradually decreasing component is a useless and often noxious "over-stimulation".

There have also been experimental attempts to determine which fraction of the exponential current excites. SUZUKI (1932, 1938) for this purpose used a HELMHOLTZ pendulum to interrupt the exponential current at any desired moment. The effective fractions, the chronaxies of the different current gradients, could in this way be determined. These times together with corresponding current strengths, his *Realschwellen*, were related to the full time values of the exponential rise as well as to the equivalent *Virtual-*

schwellen (see fig. 3). Complicated technique and unnecessarily involved calculations characterize this method. If one really were interested in what happens with the exponential type of current, direct and simultaneous recording of the form of the stimulating current and the action potential, as used below, would give the same end result far more simply and with greater precision (see *e. g.* fig. 20).

But how very much simpler is not the analysis with linearly rising currents. The current gradient is constant, and the final

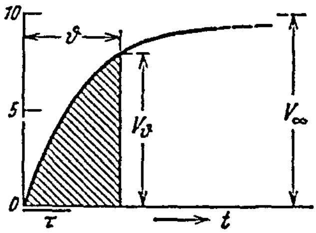


Fig. 3. From SUZUKI (1932). The exponential current with the half time value of τ reaches, when the total rising time is t, the threshold strength V_∞ (Virtualschwelle). Actually, however, it has excited already within the time ϑ which only corresponds to strength V_ϑ (Realschwelle). The effective part strippled.

value of the current at the threshold represents effective duration whilst the current strength directly gives the *Realschwellen.*

There can only be one explanation of the circumstance that, despite this, exponential currents have become the standard method in recent times. This is the generally known and often admitted fact that from the technical point of view they are so easily produced. Linearly rising currents require more elaborate preparations.

A definite technical improvement in the methods of producing linearly rising currents was FABRE's (1927) method of utilizing a valve charged by a condenser. It is nevertheless evident from his work that the whole stimulating current cannot have been linear. The same holds good with regard to the technique of KAHLSON and v. WERZ (1936), as pointed out by themselves.

Full linearity, however, is probably obtained by the *Elektronenröhren-Rheonome* of WYSS (1940) (paper not available in original), also based on the principle of discharging a condenser over a valve but with special arrangements for eliminating errors due to the valve's characteristic.

LANDOLT (1941) has used this apparatus in his research and also points out that interpretation of the accommodation curves

is simplified by the use of linearly rising currents, not in the least because lines drawn from these curves to the origo of the coordinate system represent actual current gradients. LUCAS's (1907) procedure of actually inserting the stimulating currents has not been used in his work. If this had been done his interpretation of some of the experimental results certainly would have been different.

In his experiments, with a threshold contraction of the sciatic-gastrocnemius preparation of the frog as index, LANDOLT found

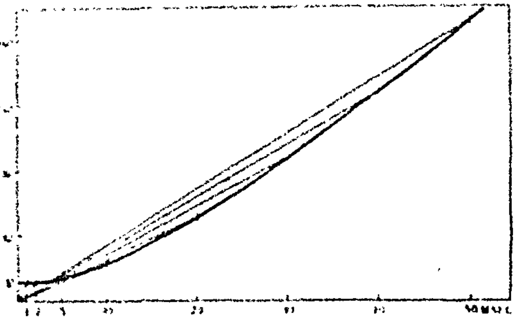


Fig. 4. From LANDOLT (1941), his fig. 2. Accommodation curve of type II. Abscissae: rising times in msec., ordinates: current strength. Rheobase = 1. The lines drawn from origo to different points of the experimentally determined curve represent current rise *not* found in the original. See text.

three types of accommodation curves. Type I is identical with the one found by LUCAS and FABRE with a minimal current gradient represented by a line through the origo (cf. fig. 2). But the same line also represents a constant current gradient. Therefore, as also pointed out by him, the rise above the first value plotted on this line (corresponding to point F in fig. 2) must be without significance for the excitatory effect. However, the same important argument unfortunately has been neglected in the interpretation of the curve of type II which is upwards concave! If the linearly rising currents are inserted (as I have done in fig. 4) one finds that up to rising times of 10 msec. the gradients successively diminish leading to a corresponding rise of the threshold. But the longer rising times, from 20—50 msec., as clearly illustrated by fig. 4, again presuppose stimulation with *higher* current gradients than those used below 10 msec. Accordingly they must have elicited contractions long before they had risen to the values found in LANDOLT's curves and at any rate could not have caused successively rising thresholds as in his presentation of the results.

As a matter of fact the real current gradient for 30 msec. on the abscissa is identical with the one for 5 msec. Thus LANDOLT's figure beyond rising times of 10 msec. is a diagrammatic construction without any physiological significance. LANDOLT's

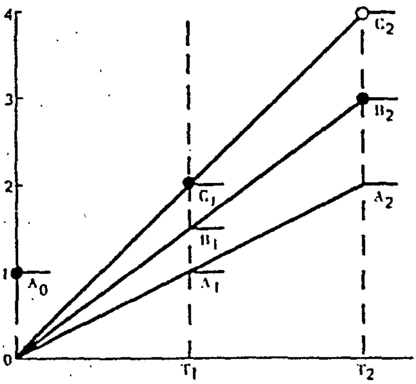


Fig- 5 a.

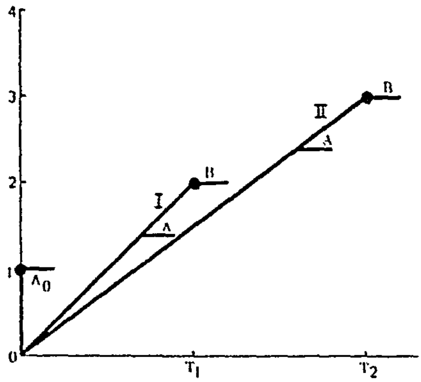


Fig. 5 b.

Fig. 5 a. Diagram showing the method of determining the thresholds with currents of fixed durations, these being given by the three abscissae 0, T_1 and T_2. The zero abscissa thus marks instantaneous current rise, or *rheobase*, requiring current strength 1 at A_0 on the ordinate. The lines drawn in full represent three linearly rising currents. The rheobase and the thresholds for the linearly rising currents are marked by the filled circles.

The rheobase is first determined. Then time T_1 is chosen and the three linearly rising currents are tested beginning with the slowest one 0—A_1. Excitation does not take place until the gradient has been increased to give 0—C_1, marked by filled circle. The next step is to choose a longer duration T_2, and again try different gradients beginning with the slowest rise 0—A_2 which does not excite. This time excitation takes place with 0—B_2. But it is clear that the next gradient 0—C_2 also must excite. It would then be a mistake to take the point C_2 to represent the current strength necessary for excitation with this gradient, as the diagram shows that it already excited at time T_1 corresponding only to strength C_1.

Fig. 5 b. Diagram showing method of determining the threshold with currents of constant gradient and variable strength. Ordinates and abscissae as in fig. 5 a. Rheobase A_0.

The current with the gradient I is increased in steps, *e. g.* 0—A, 0—B etc. This time, excitation takes place at B. Then the smaller gradient II is tested in steps 0—A, 0—B etc. until excitation occurs, this time, at B.

curve of type III, however, is correct and the successively diminishing slope of the accommodation curve confirms results obtained by, for instance, v. KRIES (1884), SCHRIEVER and CEBULLA (1938), GRANIT and SKOGLUND (1941).

LANDOLT's instructive mistake with regard to his curve type II shows that it is important not to loose sight of the fundamental observation that current gradient is the factor determining the rise of the threshold. The error is intelligible in view of the method he uses. According to fig. 5 *a* the threshold of excitation is sought

out by starting with a certain current gradient of subliminal strength. Not only in his work but quite generally in work of this character current strength is then successively increased until the threshold contraction appears. But at the same time the current gradient — owing to the design of the apparatus — is also being altered. There is nothing to criticise in this procedure as long as the experimenter all the time is aware of *what* current gradients actually have been used in the experiment. If not, he is liable to make the aforementioned mistake of using a too high current gradient when extending the duration of the stimulus. However, there remains always the objection to this procedure that the experimenter, being unaware of the real current gradients, forces too strong currents through the tissue without the excuse of direct necessity.

It is obviously more to the purpose to use an apparatus for linearly rising currents in which for any *constant* gradient the current can be driven up to any desirable height (fig. 5 *b*). Rate of rise and strength, in other words, should be independent variables.

In all the work on accommodation referred to, and independently of the manner in which the current has been produced, a muscle contraction has served as index of excitation, a somewhat surprising fact in view of the development of the electrical recording technique. This is the more remarkable as already v. KRIES (1884) realized that this index is subject to changes related to the gradient of the stimulus and thus hardly can be the constant required by theory. Criticism against this procedure has already been set forth in the introduction. HOFFMANN (1910), ISHIMORI (1912), FESSARD (1936), SCHRIEVER and CEBULLA (1938), GRANIT and SKOGLUND (1941), and BERNHARD, GRANIT and SKOGLUND (1942) have all pointed out that slowly rising stimuli may cause repetitive discharges. Both muscle and nerve have been used in these papers. BERNHARD, GRANIT and SKOGLUND (1942) have developed this observation into a critical discussion of the index for which they have also relied upon some of the results to be published in greater detail in this paper. Their interest in this question was chiefly to find a more reliable index of the excitatory effect in order to study breakdown of accommodation in relation to nerve as model sense organ. Whether a muscle contraction or a composite action potential from nerve or muscle should be used as measure of the constant excitatory effect, is not the relevant

point in their criticism. Repetitiousness of firing is just as troublesome with the one index as with the other. The important point is that the index used should be reliable. We shall see below that the methods of this work also eliminate this question of the index.

Departure of the accommodation curves from the linear form has been seen for instance in the work of GILDEMEISTER (1904), LAUGIER (1921), SUZUKI (1932), KAHLSON and v. WERZ (1936). However, it is hardly necessary to discuss this question now. It is better to return to it in connexion with my own experiments. But I shall mention some results by SCHRIEVER and CEBULLA (1938) from experiments on changes of the ionic balance of the environment of the frog's gastrocnemius caused by decalcification. In agreement with the results of SOLANDT (1936 a), the accommodation curve after this treatment was found to take a more horizontal course at the same time as the nerves began to react repetitively. Yet, the strictly linear accommodation curves, published by SOLANDT, were seen only in exceptional cases and then in non-rhythmic nerves with small accommodation constant. In the great majority of the curves the rising linear phase successively gave room for a part with gradually diminishing slope. Nerves giving curves of this type were always found to react more or less repetitively. Also LAPICQUE (1937 b) has emphasized that rhythmicity introduces complications.

GRANIT and SKOGLUND's (1941) similar results with mammalian nerves have already been mentioned. In mammalian nerves there was found a regularly recurring *breakdown of accommodation* connected with repetitive firing and muscle contractions reminiscent of contractures. BERNHARD, GRANIT and SKOGLUND (1942) demonstrated the same phenomenon when recording the activity in restricted groups of motor units with micro-electrodes. Their conclusion was that breakdown of accommodation always takes place during the time the stimulus is applied, provided that it has reached a certain level of strength (often very low in terms of multiples of rheobasic strength) and that finally there is so little accommodation left that the stimulus always excites no matter how slowly it reaches this level. The slope of the curve then becomes horizontal. The active units concerned fire repetitively in the region above the accommodation curve.

From all this work and the criticism levelled against certain phases of it, it is clear that the linear course of the accommodation curve, predicted by theory, is an abstraction for which there is

little experimental foundation unless one introduces the *ad hoc* assumption that there are qualitative differences in the reaction of the nerve, dependent upon the gradient of the stimulus, and restricts one's observations to the initial part of the curve. It would therefore seem to be both reasonable and necessary to analyse these questions by means of experiments designed to throw light upon the discrepancies referred to, and, to this end, to make use of methods leaving more room for demonstration and less for inference than those based merely on observation of the ultimate effect, a threshold muscle contraction.

2. Constant Currents.

Work of this character is, of course, closely allied with the study of the effect of constant currents, this already because of the fact that the type of stimulus used, both previously and in this paper, implies that the linearly rising current remains some time at the level reached at the moment when excitation takes place. With my apparatus it is actually possible to interrupt the current at any moment or to let it gradually slope downwards, but if this is done the analysis is complicated by "opening tetanus" *following* immediately upon the closing phenomena. For this reason I too have chosen to let the current continue for some time at the plateau level reached at the moment of excitation. For this choice there is the further motive that a current form of this type more closely imitates the generator potentials (see above, BERNHARD, GRANIT and SKOGLUND, 1942) which it is one of the purposes of this paper to imitate. As pointed out above, slowly rising potentials, often continuing at plateau height, characterize certain sense organs.

It is clear that also from the point of view of accommodation the effects of constant current deserve close attention. The work of DUBOIS-REYMOND (1849), PFLÜGER (1859) and other exponents for classical electrophysiology need not here be recapitulated. A very good review of the old work has been given by GOTCH (1900). I shall only mention some recent experiments of particular interest for the theme of this investigation.

The cathodal depression following application of a subrheobasic current was studied in an important contribution by ERLANGER and BLAIR (1931) by the method of following the course

of excitability, consequent upon subrheobasic excitation, with test shocks at suitable intervals. At the cathode was found the well-known decrease of threshold dropping to a steady plateau lasting some 1—2 msec. and succeeded by an increased threshold. This, the cathodal depression, set in at a relatively fast rate and then reached its maximum level more slowly. The depression would make the nerve respond to closure at the cathode with a single action potential only. But, as pointed out above, an isolated frog nerve may also respond repetitively to a rectangular constant current, PFLÜGER's closing tetanus. GOTCH (1900) spoke of a state of prolonged excitation at closure. ERLANGER and BLAIR (1936) showed that in nerves reacting in this manner the cathodal depression developed very slowly or was absent. In this manner accommodation is related by ERLANGER and BLAIR to the cathodal depression. A uniform technique with a polarizing current of 80 % of the rheobase and a test shock following after 10 msec. thus provides them with an objective measure of the degree of cathodal depression and thereby of accommodation — in this sense.

This method was used in the above mentioned experiments by ERLANGER and BLAIR (1936) on frog nerve in which they found accommodation, measured by this index, greater in motor than in sensory nerves and also noted a tendency to repetitiousness in the latter. This tendency was obvious both with regard to the threshold for repetitiousness and the maximal amount of repetition.

The same method was used by PARRACK (1940) for the analysis of changes of excitability in frog nerve due to changes in the external milieu. His index was the half maximum alpha response observed on the cathode ray oscillograph. He came to conclusions which with regard to the effect of Ca-ions on accommodation were opposite to those agreed upon by previous workers, quoted above. He also concludes that accommodation measured in circulated nerves is negligeable, a rather surprising result in view of measurements of accommodation in intact human nerves through the skin (see *e. g.* SOLANDT, 1936 a, GRANIT and SKOGLUND, 1941), measurements which are easily confirmed and always show relatively steep accommodation curves. It is therefore improbable that his methods and results can refer to the phenomena studied by other workers in a different manner.

ROSENBLUETH (1941 a, b) has studied the electrotonic changes in excitability in myelinated cat nerves, both excised and *in situ*, and

points out that mammalian nerves react repetitively to current strengths from 1.5—3 times the rheobase. He does not in recording from nerve differentiate between motor and sensory nerves. The most important result of his work, however, is an experimental criticism of PFLÜGER's (1859) simplified scheme of excitability changes under and around the stimulating electrodes. Dependent upon strength of the stimulating current and the interpolar distance very complex combinations of local troughs of increased or decreased excitability appear in both the anodal and the cathodal regions. One is forced to conclude that we do not yet possess enough knowledge about the electrotonic excitability changes to make a discussion of the significance of these changes profitable. But their complexity makes one hesitate to identify the concepts of accommodation, derived from two as different methods as, on the one hand, those of workers using exponential or linearly increasing currents, say, in the manner of HILL and others, and the method of ERLANGER and BLAIR and PARRACK on the other hand.

The work of FABRE (1934, 1936 a, b, 1938) also indicates a very complex state of affairs with regard to excitability changes caused by the constant current. Of particular interest is an experiment (1936 a) in which he compares the method of testing with brief shocks in the manner of ERLANGER and BLAIR with a testing method utilizing linearly rising currents. FABRE first demonstrates how the excitability, tested with single shocks, rises under a catelectrotonus and sinks upon interruption of the constant current. Also under an anelectrotonic state there are opposite effects during and after closure of the current. But if then the anelectrotonic excitability is tested with linearly rising currents, FABRE finds that his *pente limite* falls, *i. e.* accommodation diminishes and *remains* diminished after interruption of the constant current. The same holds good for the rise of the *pente limite* in catelectrotonus. The decrease of accommodation in anelectrotonus has been confirmed by SHANES (1940).

FABRE's view, that excitability tested with slowly rising currents refers to a physico-chemical system with other properties than those found when brief test-shocks are used, further emphasizes the necessity for extending a study of the effects of linearly rising currents to an analysis involving separation of motor and sensory nerves.

In this review of accommodation a number of problems have

been neglected which only in an indirect manner are connected with the present work. However, some of them will be mentioned in connexion with the analysis of my own results.

One of the questions discussed in the literature on accommodation refers to whether the time constant of accommodation varies in parallel with the time constant of excitation (chronaxie) or not. Supported also by SOLANDT's (1936 a) experiments and the results of FABRE (1931 b) HILL claims that the two constants can vary independently whereas LAPICQUE (1937 b) holds that they vary in parallel. The work of LIESSE (1938 a, b) should also be mentioned in this connexion as supporting HILL's conclusions. SCHRIEVER and CEBULLA (1938) present experimental results in favour of the conclusion that the constants undergo similar changes in non-rhythmic nerves but become independent in rhythmic nerves. Some papers on the effect of changes in the milieu of the excitable structures are better mentioned in connexion with my own results.

Both chemical and electrical models have been described in attempts to understand the processes behind accommodation (FABRE, 1931 b, LAPICQUE, 1926, SCHRIEVER, 1933, EICHLER, 1939 a, b). For these the reader is referred to the papers quoted.

Technique and Procedure.

1. The Stimulator.

For the stimulator used in this work the author is indebted to the physicist of this laboratory, Mr T. Helme. It was designed for delivering iterated current pulses of variable form and strength rising and falling linearly. It is therefore unnecessarily complicated for this work in which only single stimuli have been used. The arrangement for automatic iteration, in the part labelled II in the diagram of the apparatus (fig. 6), was introduced with a view to a planned extension of the programme.

The apparatus (fig. 6) may schematically be divided into the three parts marked on the diagram. Stimulation is started by pressing the contact A whereby the "charging circuit" I puts the "stimulating circuit" III into operation, at the same time blocking the "discharging circuit" II by means of which the condensers of I are emptied when the contact at A again is broken. The stimulation circuit III is activated by the rising charge of the variable condenser battery in I removing a block on the grid of III at a rate dependent upon the capacities chosen. The preparation is thus stimulated by current from the valve V_3.

The charging circuit I: In accordance with this principle the charging circuit consists of a condenser battery of variable capacity coupled to a valve (V_1) keeping the strength of the charging current constant. This being constant the charging rate is a function of the capacity chosen. The charging voltage rising at a constant rate charges the grid of V_3 thus setting up current in III, a fraction of which, as pointed out above, stimulates with the rate of rise determined by the factors mentioned. The strength of the stimulating current is independently determined by the choice of different final potentials in the condenser battery by means of the variable voltage of the anode battery of V_1. This redistribution of current in the apparatus leading to stimulation, as stated, is started manually at A.

The discharging circuit II: This consists of two valves with resistances. V_2 is the discharging valve. Closure of contact A blocks this circuit distributing the energy into the channels leading to stimulation, as described above. Blocking of II can be stopped automatically or by breaking contact A at the moment when the potential of the condenser battery in I has been charged to maxi-

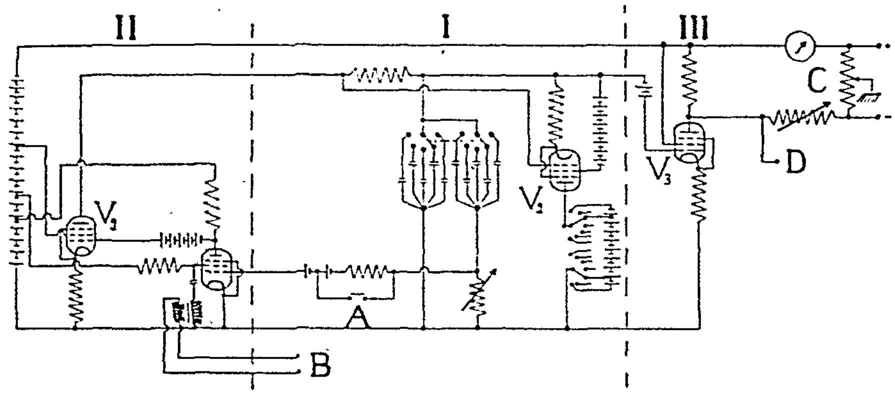


Fig. 6. Diagram of stimulating device. See text.

mal potential. In this work manual operation of contact A has been used throughout. At this moment the condenser battery discharges into II with a linear fall of the stimulating current in III which with respect to its gradient is a copy of the rise as shown in fig. 7. The circuit can be adjusted so that the fall immediately succeeds the maximum of the rise, as in fig. 7 *a*, or later, after any desired time of maximal stimulation at plateau height, as in fig. 7 *b*. This is the type of stimulus used throughout in this work.

Figs. 7 *c* and *d* illustrate the apparatus operated by an ordinary neon-stimulator coupled at B of II.

The stimulating circuit III: This consists of a pentode with high internal resistance to compensate for variations in the preparation and the electrodes. C is the output. Current strength is measured on the ammeter, also inserted in the diagram. For absolute values the readings are corrected for the resistances in the circuit. The shunt of 10.000 ohms, connected to earth, is a Bishop compensator (1927) for elimination of shock artefacts. For this purpose the leads are also screened and capacitively balanced. In the lead to the cathode has been placed a resistance of not less than 0.1 $M\Omega$.

The apparatus described in this section evidently satisfies the criteria raised in the Historical section, p. 18. Linearly rising currents of any desirable gradient are obtained and for every constant gradient chosen the strength of the current can be pushed

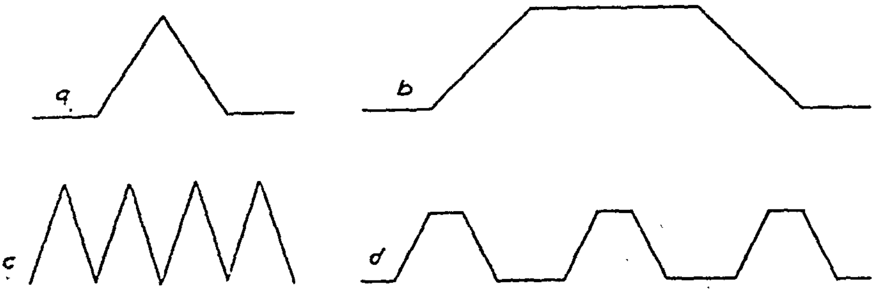


Fig. 7. Some typical current forms given by the stimulator.

up to any desirable value. The apparatus can also be worked by keeping the duration of the rise constant and varying strength and rate of rise.

2. The Recording.

In order to mark the moment of stimulation and to record the form of the stimulating current the latter was led from point D in the diagram (fig. 6) to a directly coupled amplifier capable of shifting the one beam of the double cathode ray oscillograph. With this arrangement the deflexion of the beam could always be adjusted so as to enable accurate recording of the form and duration of the rising stimulus. However, proportionality between the size of the deflexion and the strength of the stimulating current was not in the first instance a goal in these experiments — though possible to obtain — since, for this purpose the ammeter in the circuit III gave the information wanted.

The main principle has been not to rely on mere adjustments of knobs for production of the stimulating current but in each record to measure the form strength and duration of the exciting impulse.

In the first experiments a neon lamp coupled to the 50-period A. C. mains was used for time-marking. In some of the figures 20 msec. are thus marked by spots along the edge of the film. Later the beam, recording the stimulating current, was made intermittent with the aid of a Philips frequency generator recali-

brated at the laboratory. Frequencies from 100—1 000 per sec. were used dependent upon the speed of the rising stimulus. Before setting the frequency of the generator it was always photographically calibrated to the 50-period A. C. mains, and, in addition, at the end of long lasting-experiments a further calibration was carried out. The photographic records gave a correction factor by means of which the accuracy of time recording was further improved.

For photography two cameras have been used, one for running records on a rolling film of 35 mm bromide paper, the other one for standing records of a deflexion of the sweep of the oscillograph.

For brief rising times of the stimulus it was necessary to synchronize it with the sweep in such a manner that both beams of the cathode ray, the one recording the stimulus the other one the impulses through the condenser-coupled amplifier, were carried simultaneously once over the screen of the tube. This process was reproduced by the camera in the scale 90×90 mm. On account of the curvature at the edge of the cathode tube some deformation takes place in the periphery of the picture but, as this distortion affects both beams equally, it is without influence on the measurements. Deficiencies in the linearity of the sweep become noticeable towards the end of the deflexion of the latter, but are, of course, immaterial when the processes are related to the time-marking. A small correction has been necessary on account of a minimal difference in level of the two cathode beams.

The amplifier for the impulses was 4-stage condenser push-pull-coupled, and, as stated, connected to the second beam of the double cathode ray oscillograph.

3. The Preparation.

For most experiments decerebrate cats have been used; in some cases also animals which had received intraperitoneally 0.4—0.5 cc Dial Ciba per kg body-weight. The spinal cord has been opened in the lumbar region and the sensory and motor roots on one side have been dissected free and severed near the cord. The sciatic nerve has been severed or ligated just below the knee and a few cm of either the peroneal or the popliteal branch have been isolated for the stimulating electrodes. As much as possible of the rest of the nerve has been left with intact blood supply. When the saphenous nerve has been stimulated it has been similarly prepared.

In experiments with a preparation of this type the impulses have been recorded from either the motor or the sensory root.

In another type of experiment action potentials have been recorded from the tib. ant. muscle which then, as well as possible, has been isolated from the rest of the musculature and attached to a BROWN-SCHUSTER spring myograph. The cat has then been fixed in the usual manner with drills through the condyle of the femur and the lower part of the tibia. In these experiments the sciatic — severed or ligated above the electrodes — has been stimulated.

In all experiments the animal has been in a metal box in which high temperature and humidity has been kept up by heating a layer of water covering the bottom of the box. The temperature of the animal is around 38°.

The stimulating electrodes have been daily chlorinated silver rods, 3 cm$\times$1 mm, inside a glass tube containing Ringer solution. Contact to the nerve has been through cotton wicks. In these experiments polarization has been negligeable at the current strengths employed, as repeatedly has been checked. The electrodes have been placed with cathode towards the conducting part of the nerve at an interpolar distance of 15 mm. After each experiment the stretch of nerve from stimulating to recording electrodes has been measured on the isolated nerve. The recording electrodes for nerve action potentials have been small platinum hooks, with or without a covering layer of moist cotton, placed at an interpolar distance of about 5 mm. For recording of muscle action potentials micro-electrodes consisting of silver pins have been used drawn out with glass to a fine point of about 25 μ. Recording has been monopolar with the micro-electrode stuck into the muscle and another electrode on the drill through the bone.

Results.

I. Motor Nerves.

1. General Results.

In the experiments to be described the stimulating electrodes have been on the peripheral sciatic, as mentioned in the section on Preparation (p. 27), and the recording electrodes on a filament of a motor root.

The advantages of this arrangement are several. The stimulus activates a relatively intact and well circulated stretch of nerve which — as the experiments will show — satisfies the condition that the results should be repeatable and constant. At the region where recording takes place the root filaments can easily be divided into highly restricted units. The sympathetic fibres, on account of their special properties with regard to threshold and time-constant (WYSS, 1934), do not enter into the question.

When leading off from a filament the response consists of a relatively simple spike of high amplitude. In fig. 8 this spike is shown for a number of currents of different gradient and strength, marked in the figure text. We need not now discuss the question as to whether the spike represents a single fibre or synchronized activity in a restricted group of fibres, but may regard it as a highly restricted, reproducible unit. The records show how the latent period of this spike is lengthened when the rate of rise of the stimulating current is diminished. In fig. 8 the strength of the stimulus is proportional to the deflexion of the beam recording stimulus form. Therefore the figure also shows that the downwards *de*creasing rate of rise of the stimulus, lengthening the latent period, is compensated for by *in*creasing current strength. Thus accommodation is clearly illustrated by this figure.

The records of fig. 8 have also been selected to emphasize some other effects.

We note in record *d* that the current strength necessary for the discharge of the spike is far below the final value reached in this experiment. Thus an unnecessarily strong stimulus has been used. Actually a current of a strength corresponding to

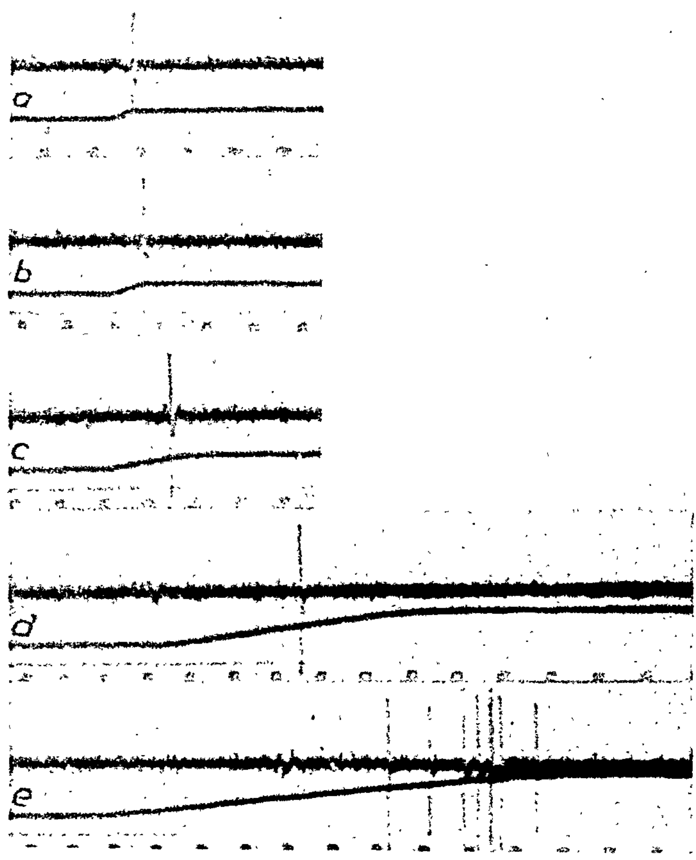


Fig. 8. Leads on filaments of motor roots. Sciatic nerve stimulated. Time in 20 msec. Spike size about 100 μV. For each record rising time, final current strength, latent period and strength at latent period are as follows:

a:	6 msec.	28 μA.	5 msec.	24 μA.
b:	15 msec.	40 μA.	12 msec.	32 μA.
c:	35 msec.	52 μA.	27 msec.	40 μA.
d:	120 msec.	120 μA.	60 msec.	60 μA.
e:	200 msec.	170 μA.	120 msec.	102 μA.

that reached at the moment of discharge — or even a little below it — would have been sufficient. In the next record (*c*), where at a given gradient the stimulating current has been allowed to rise still further, one can see that this additional increase of stimulus strength by no means is negligeable

from the point of view of nerve excitation. As long as the rise is continued the nerve responds iteratively, as also predicted by HILL's theory. This phenomenon can be seen at all gradients though in this case merely illustrated at the slowest rate of rise. We shall return to it below. However, by properly

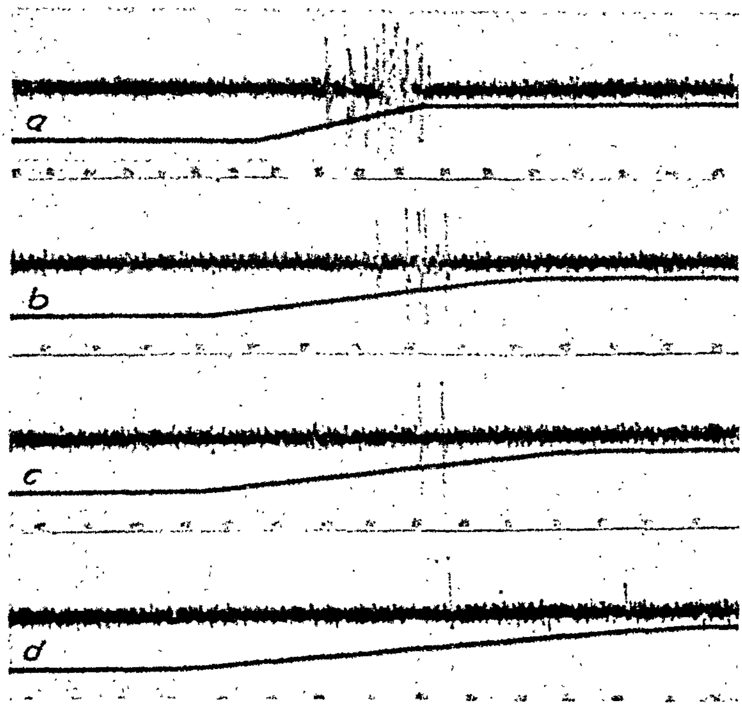


Fig. 9. Stimulation of sciatic nerve with successively slower gradients rising to the same final value. Time in 20 msec. Rising time, final current strength, latent period and strength at latent period are as follows:

a: 80 msec. 120 μA. 35 msec. 53 μA.
b: 120 msec. 120 μA. 65 msec. 65 μA.
c: 170 msec. 120 μA. 100 msec. 70 μA.
d: 200 msec. 120 μA. 115 msec. 70 μA.

adjusting the stimulus the motor nerve may be made to discharge a single spike, also to very slowly rising stimuli, as shown by record *d* of fig. 9.

In fig. 9 a different experimental principle has been followed. The final value to which the current has been allowed to rise has been kept constant. This constant strength has been chosen to correspond to the activation of a single spike at the slowest gradient. The gradients rise from below upwards. Thus the threshold is exceeded in the same direction, for each record by a greater

amount, suprathreshold excitation being maximal in *a* where the gradient is maximal. Frequency of repetition increases in the same direction. Somewhat in this manner a generator potential in a sense organ might increase its effect by a variation of its rate of rise.

Again in fig. 10 the gradient has been kept constant in all records but the final strength of the current has been increased

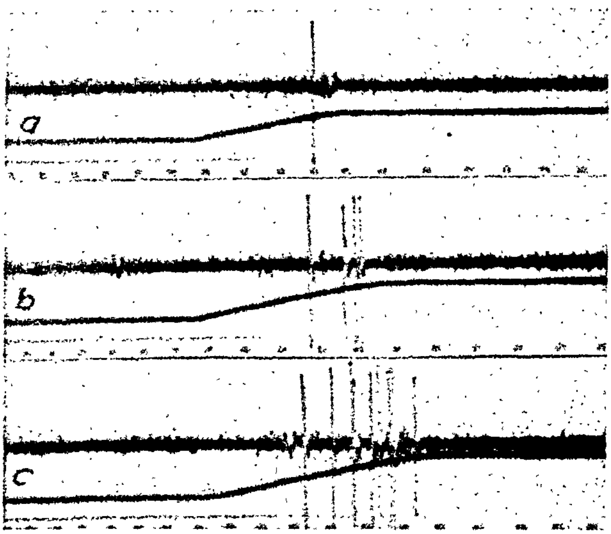


Fig. 10. Stimulation of sciatic nerve with stimuli of constant gradient rising to different strengths. Time in 20 msec. Marked as in figs. 8 and 9.

a: 80 msec. 100 μA. 60 msec. 75 μA.
b: 100 msec. 124 μA. 60 msec. 75 μA.
c: 130 msec. 160 μA. 58 msec. 72 μA.

from *a* to *c*. In *a* a current strength of 100 microamp. has just been a little above the strength necessary for a spike at a latent period of 60 msec. In *b*, at the same gradient, the nerve discharges after the same latent period. But the increase of stimulus strength from 100 to 124 microamp. has led to three additional spikes. The same principle is illustrated in record *c* showing a further increase in current strength, to 160 microamp.: practically unaltered latency of the first threshold spike and iteration during the rising phase of the current.

These experiments serve to illustrate the principle of the method of determining the threshold that I have used for the plotting of accommodation curves. The duration of the rise of the stimulating current is obtained from the photographic records, and the

final strength of it is read from the ammeter. The stimuli are then plotted in a coordinate system with duration as abscissae and strength as ordinates (fig. 11). From the origo to these points are drawn the straight lines corresponding to the different rates of rise of the stimuli. Upon each rising stimulus is inserted the value for the latent period of its first impulse. The points so obtained are

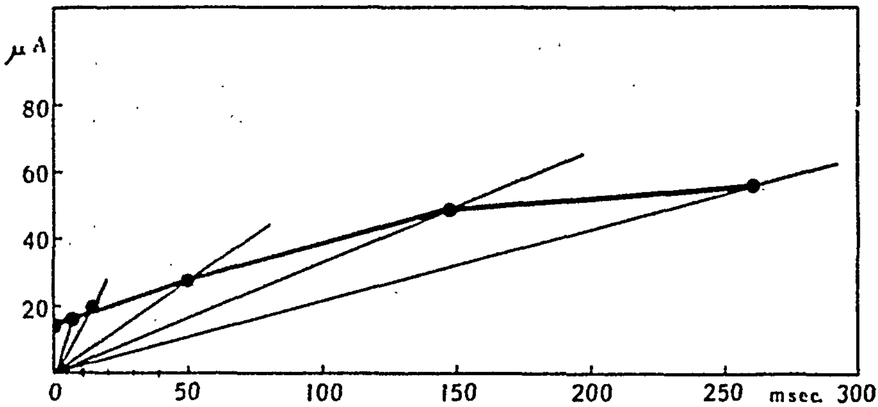


Fig. 11. Showing how an accommodation curve (motor fibres) is plotted. Ordinates: absolute current strength in microamp. Abscissae: rising times in msec. The thin lines from origo represent the rising stimuli. These are first drawn and then the latent periods, marked by filled circles, are inserted on the stimuli. The points are joined by a thick line.

joined by a line as illustrated by fig. 11. These points, given by the latent periods and combining to the accommodation curve, represent ordinates just around the current strength necessary for the first spike. Below in chapter 2, when separately presenting results for very brief stimuli, I shall return to the question of the significance of this ordinate.

This method of determining the thresholds necessary for the accommodation curve offers great advantages, apart from being more accurate than any method hitherto used. In experimental practice the results are quickly and simply obtained by selecting a number of current gradients and for each of them increasing current strength till spikes appear on the screen of the cathode ray. It is immaterial whether one or several spikes are seen, the threshold being always determined by the latent period of the first spike. One need not therefore repeat stimulation many times in order to hit on the precise threshold.

With spikes from a great number of different fibres, as illustrated in fig. 18, the first spike represents the latent period for

the fibres with lowest threshold. Thus, in general, the measurements will refer to motor alpha fibres.

The accommodation curves obtained from 12 cats in this manner are very similar and the curve of fig. 11 may be taken as typical. The ordinates have been obtained from the ammeter as strength of current through the stimulating electrodes. The actual amount of current passing through the nerve is, of course, unknown, and so the ordinates merely represent relative current strength.

In order to compare different experiments it has been necessary to use multiples of rheobasic strength, as in fig. 4. In the accommodation curves which I have plotted in this manner (*e. g.* figs. 12, 13 and 14) each ordinate value shows how many times it has been necessary to increase the rheobase in order to elicit an impulse when the current has risen during the time marked by the corresponding value on the abscissa. Alternatively one could plot these ordinates against current gradient. But a curve obtained in this manner would not picture the results as well as the plotting used.

Fig. 11 shows that with decreasing rate of rise of the stimulus the increase in threshold, to begin with, is proportional to the time it has taken the current to reach threshold strength. Gradually, however, this relation breaks down and the curves tend to flatten out. When the curves reach this more or less horizontal level, even slower stimuli than the ones used are capable of exciting the nerve, provided that they rise to the strength marked by this level.

However, extremely low gradients have but rarely been used. These hardly mirror any physiological processes, to be judged by the fact that the thresholds then begin to undergo great and uncontrollable variations.

The uniform and well reproducible results with moderate current gradients thus show that the normal circulated mammalian motor nerve possesses an accommodation curve which for short rising times is relatively steep (for precise values see below p. 37) but that breakdown appears with gradients corresponding to as moderate current strengths as 3—4 times the rheobase. The mammalian motor nerve prepared *in situ* in this manner therefore is lacking the *pente limite*, the minimal current gradient below which no excitation takes place, known from the excised frog nerve.

Having described the general type of mammalian accommodation curve for motor nerves we shall now enter into a more detailed analysis of it.

2. Limits of Variation.

The experiments have shown that the mammalian preparation is stable and gives reasonably uniform results within 2—4 hours from the moment of isolation of the nerve, the last stage in the preparatory operations. However, this presupposes that the animal

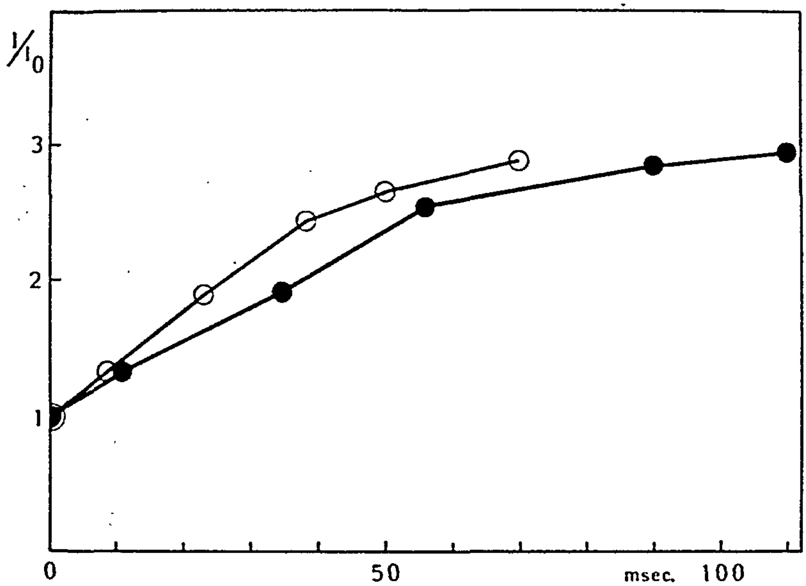


Fig. 12. Accommodation curves (motor fibres) from single experiment, taken at interval of 2 hours. First set of values: open circles. Second set of values: filled circles. Ordinates: current strength divided by rheobase (I/I_0). Abscissae: rising times in msec.

is in a good state after decerebration and other operations. When, in some experiments, variable results have been obtained after half an hour or less, this has been a reliable indicator of an incipient collapse of the preparation, soon to be followed by disturbed breathing and other signs foreshadowing an early exitus.

It has not been possible to find a definite difference between decerebrated animals and those which received the moderate dose of dial used in some experiments.

It is true that some variations occur from measurement to measurement on the same preparation but they are of no significance for the general course of the curves. In fig. 12 there are two accommodation curves recorded at an interval of about 2 hours. A variation of rheobase had occurred but whether it depends on physiological factors or on a resistance change is difficult

to say. But by expressing the ordinates, in this curve and in those to follow, in multiples of rheobasic strength we find a basis for comparison. The two curves of fig. 12 are then practically identical both with respect to initial slope and breakdown.

Experiments on different cats have given curves of slightly different types. The most common type is the one shown in fig. 13.

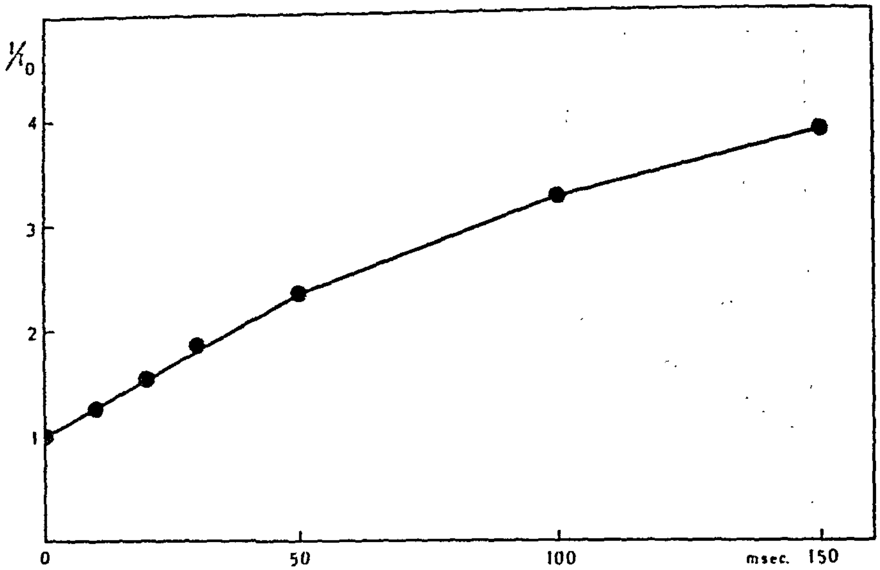


Fig. 13. Accommodation curve for motor fibres. Common type with initial linearly rising portion.

Fig. 14 illustrates another variant lacking the linearly rising phase and having a successively diminishing slope. But as a rule there is a linear rise up to values corresponding to rising times of about 50 msec. As will be shown in fig. 16 (p. 41) the curves for very brief times also have the initial linear phase.

Whereas it is difficult to refer breakdown of accommodation to a definite point on the curve it is nevertheless clear that the horizontal portion represents an ordinate of 3—4 times the rheobase. This is the average for the great majority of curves.

Below I shall compare these accommodation curves with those obtained from sensory nerves. It will then become apparent that the differences are of an order of magnitude that is clearly reproduced already by the graphic constructions. But if in addition a figure is wanted to express this difference schematically, this can be obtained by a rough approximation of the angular coefficient of the initial rise of the accommodation curves. In order to compare

the result with those mentioned in the Historical section I have taken the inverse value of the slope, as done by HILL and his collaborators in calculating their constant λ.

In table 1 (Motor) this factor is calculated for my experiments. Despite the fact that these values are approximations they vary within relatively narrow limits.

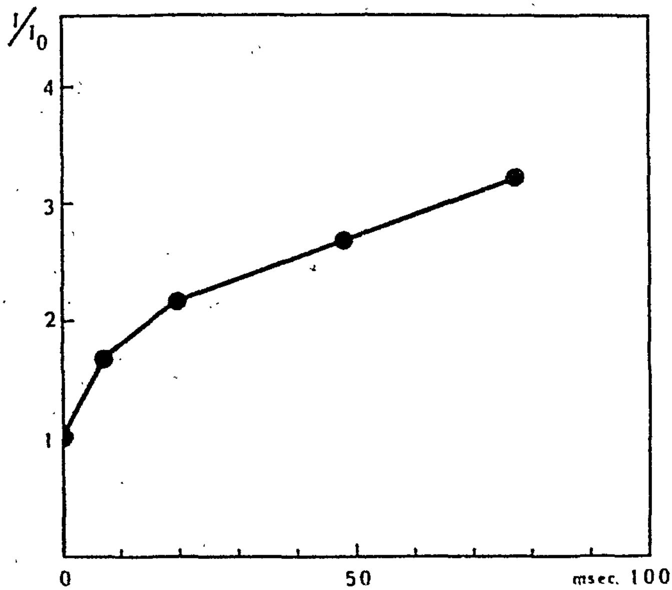


Fig. 14. Accommodation curve for motor fibres. Less common type with successively diminishing slope.

Table 1.

Experiment	Motor	Sensory
1	50	—
2	31	200
3	30	100
4	—	270 (*saphenous*)
5	32	160
6	38	132
7	44	115
8	30	over 1,000 (*saphenous*)
9	41	—
10	45	90
11	30	90
12	60	200
13	34	—

The results differ in this respect from those obtained by GRANIT and SKOGLUND (1941) and those of BERNHARD, GRANIT and SKOGLUND (1942) with a threshold muscle contraction as index. This index is certainly the main factor accounting for the difference. I shall return to this question below (p. 46) in connexion with the experiments on muscle action potentials. But the placing of the stimulating electrodes also is of importance. GRANIT and SKOGLUND found that the distance from the cut end of the nerve played a rôle. Their experience has now been utilized in order to avoid this source of error.

The constant distance between the stimulating electrodes in all my experiments has also served to standardize the results. SCHRIEVER (1932) has analyzed interelectrode distance with reference to the *Einschleichzeit*. He found an increase of this time with increased interelectrode distance, especially well marked below values of 10 mm. Between 10—30 mm. the effect was less noticeable. Similar results have been obtained by SOLANDT (1936 a). Thus, for instance, the value of λ at 2 mm. was 75 % below that at 30 mm. Between 6—30 mm. there was no regular difference. The surface under the electrode, varied from sharp edges to 1 cm., did not influence λ. SCHRIEVER (1932) also reports irregular results with monopolar stimulation, an experience confirmed by GRANIT and SKOGLUND.

With a nerve in good condition, circulated from the intact portion remaining in the tissue, there should not be found any sudden reversals of excitability nor any opening discharge to currents of moderate strength.

3. Brief Rising Times.

With the aid of the single sweep arrangement it has been possible to analyse the brief rising times corresponding to the fast gradients in the beginning of the accommodation curve.

It is obvious that physiological variations as well as technical errors in measuring etc. become relatively more important with fast gradients of stimulation. For the relatively slow gradients hitherto studied it was found possible to use threshold determinations based on the latent period. But for brief rising times it has been necessary to fall back upon the classical method of determining the threshold by taking a number of subliminal and just supraliminal readings around the limen. The variation at the

threshold, described by BLAIR and ERLANGER (1933) tends to make the measurements more difficult than for slower gradients. Considering that experiments published by *e. g.* SOLANDT (1936 a) indicate a departure from the linear slope of the accommodation curve for very brief rising times, it has been held to be of particular importance to obtain accurate figures for these gradients.

The different factors can be profitably discussed in connexion with some records taken with the sweep circuit.

Fig. 15 is intended to show how already from the beginning accommodation depresses the threshold at the slightest delay of the rising stimulus. In record *a* the small instantaneous shift in the beam recording the stimulus corresponds to a just subrheobasic stimulus of 12 microamp. In the other beam, recording the effect from the nerve, there is only a small stimulus artefact of the kind seen when compensation is insufficient. But it is so small that it does not interfere with the analysis of the action potential, as will be seen below. In record *b* the instantaneously rising current at 15.2 microamp. already is supraliminal, to be judged by the action potential that now follows. We then proceed to diminish the rate of rise of the current, keeping its strength constant at 15.2 microamp. In the records *c* and *d* can be seen the effect of changing the duration of the stimulus rise from instantaneous to respectively 0.5 and 2.0 msec. This clearly reduces the effect of the stimulus, as evident from the successive diminution of the action potential. The same current, of strength 15.2 microamp., became subliminal when rising during 3.5 msec., and in order to obtain the response of record *c* of the same figure it was then necessary to increase the current strength to 18 microamp.

The procedure of taking the latent period of the first spike for measuring thresholds, so convenient with slower gradients, is not accurate enough for brief rising times. What is first, we may ask, the probable error in measuring the time markings of 1.0 msec. at the speeds possible with the sweep? It was found to be 0.25 msec. It is also necessary to correct for the conduction time which for these low threshold motor alpha fibres has been taken as 90 m. per sec. This time, including a minute difference in the level of the two beams has been, on an average, 1.25 msec. The degree of accuracy that can be claimed for the measurements of the latent periods is limited by these approximations.

In record *c* of fig. 15 the corrected latency is 2.0 msec. A sti-

mulating effect from that part of the current which is horizontal cannot be excluded. In order to be certain that the results are not due to the limiting conditions set by the strength-duration curve it is necessary that the measurements should not be extended to rising times below 2.0 msec. (cf. WYSS, 1934). Even when this criterion is observed the physiological threshold variations are

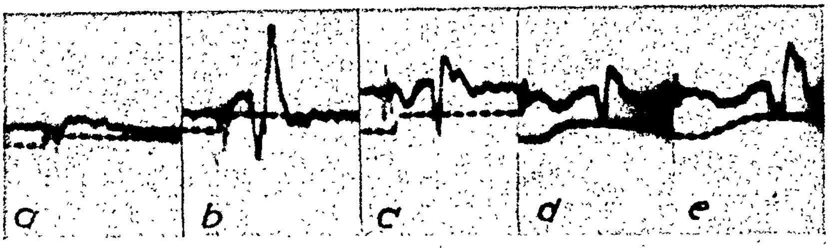


Fig. 15. Leads on motor root. Stimulation of sciatic nerve. Time in msec. a and b, instantaneously rising current; c, d, and e, brief rising times. Full description in text. Size of spike in b about 150 μV.

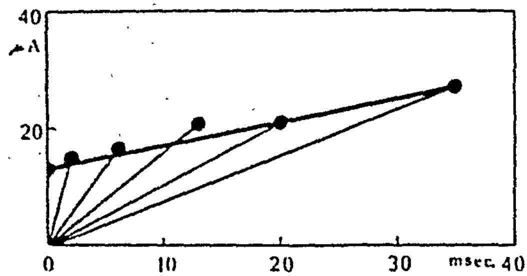
great enough to interfere with the accuracy of the measurements.

Some results of the analysis of the brief rising times may now be presented. These are shown in fig. 16, from two different experiments. The index has been a constant spike of threshold size. There are some variations in the distribution of the values. But the curves in their initial part do not differ systematically from their later course. The increase in the threshold, as a rule, is linear from the beginning as in fig. 16 *a*. But there have also been cases (fig. 16 *b*, cf. also fig. 14) in which the whole accommodation curve gradually diminishes in slope from the beginning.

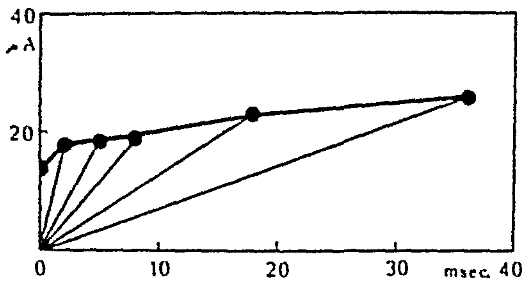
There are still some facts worth noting in connexion with the latent period of the response.

Fig. 17 shows a number of records from a single experiment. The rheobase was first tested with 24 microamp. which was definitely subliminal. The threshold was found to be around 28 microamp. Then the rheobasic current was increased to 40 microamp. This led to the expected effect: a large potential caused by synchronized elements. *At the threshold* (record *a*) the latent period is 1.75 msec., *for the supraliminal stimulus* (record *b*) only 0.25 msec. This difference represents a difference in the rate of rise of the polarization potential, known from work on instantaneously rising currents (see *e. g.* EICHLER, 1939 a and b).

Now there is no reason to assume that this shortening of the latent period with increased stimulus strength would be demonstrable only with instantaneously rising stimuli provided that the rising times are brief. That it also is present with linearly rising currents is shown by records *c* and *d* of fig. 17 in both of which



a, accurate threshold measurements for brief rising times showing that the curve rises linearly from the beginning.



b, accurate threshold measurements for brief rising times showing that there is a gradually diminishing slope from the beginning.

Fig. 16. Accommodation curves for motor fibres.

the gradient is the same. In *c* at the threshold (28.8 microamp.) the latent period is 6 msec., in record *d*, at stimulus strength 40 microamp. the latency is reduced to 5 msec.

If the stimuli are rising slowly this difference in latent period as a function of stimulus strength, if at all present, is difficult to demonstrate with the methods used. At any rate it would be a negligeable fraction of the whole latent period. Record *e* shows an impulse elicited, at threshold strength 32 microamp., by a stimulus rising at a certain gradient. The latent period is then 19 msec. In record *f* stimulation has been continued until reaching 70 microamp. at the same gradient. Again the latent period of the first spike (used in my calculations of the accommodation curve) is 19 msec. Thus, if the threshold for this gradient be calculated as the strength corresponding to a latent

period of 19 msec., it will be identical in both cases, that is, approximately 32 microamp. The experiment shows that the latent period as a basis for calculating threshold current strength in the manner suggested in chapt. 1, is satisfactory.

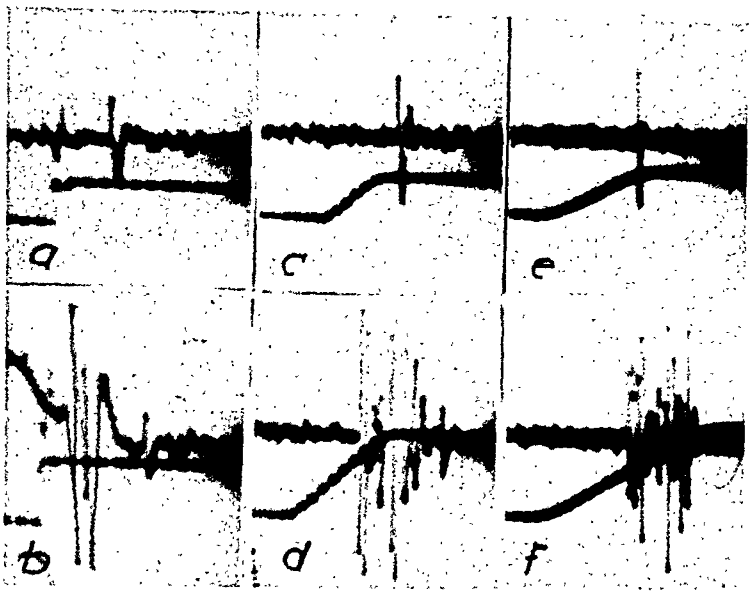


Fig. 17. Leads on motor root. Stimulation of sciatic nerve. Brief rising times; a and b, instantaneous, c and d, very fast gradient, time in msec; e and f, less steep gradient, time in 2 msec. Explanation in text. Size of spike in a about 50 µV.

4. Records from Whole Motor Root and from Muscle. Grouped Action.

In fig. 17, record *b*, an instantaneously rising suprathreshold current was found to lead to an augmented spike potential due to the activation of fresh fibres discharging iteratively.

We shall now analyze the corresponding phenomena for slowly rising currents, when a greater number of fibres are allowed to contribute to the response. Some typical pictures with leads on the whole root are shown in fig. 18. In record *a*, a just suprathreshold stimulus activates a number of large iterated spikes, just as with instantaneous stimulation. With a somewhat slower gradient, record *b*, large spikes are also obtained, but it has been necessary to drive the current a great deal above the threshold: the large spikes, seen in the record, come after a latent period corresponding to a very much stronger current than the one necessary for small spikes. A still slower gradient, record *c*, is

incapable of eliciting any large spikes despite greatly increased current strength; there is only a slight increase in the size of the spikes. This state of affairs is still more prominent with a still slower gradient, record *d*.

What is the significance of the occurrence of large spikes? They cannot represent fibres of large diameter, since these, as

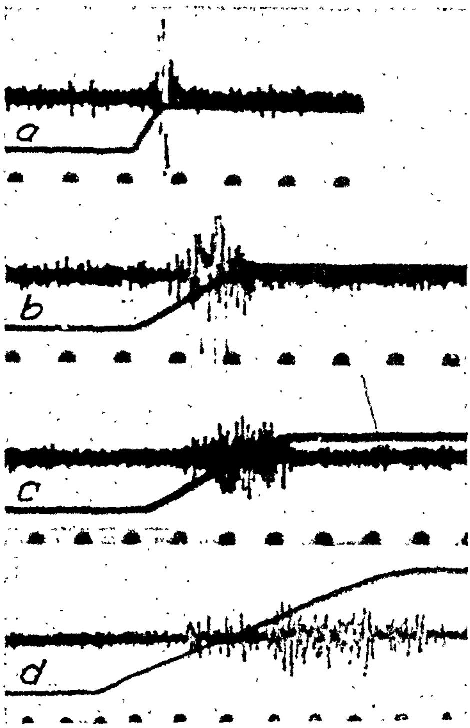


Fig. 18. Records from motor root showing synchronized spikes to suprathreshold stimuli of relatively fast gradients. See text. Values below marked in order as in fig. 8.

a.	10 msec.	12 μA.	7 msec.	8.4 μA.
b.	40 msec.	24 μA.	16 msec.	9.6 μA.
c.	55 msec.	28 μA.	25 msec.	12.7 μA.
d.	150 msec.	48 μA.	45 msec.	14.4 μA.

is well known, have low thresholds and therefore should be the first to appear when the stimulus reaches threshold strength. Instead we see that at such strengths there are only small spikes, and that the large ones require additional current strength. They must therefore be due to synchronized activity in several fibres. The explanation of the fact that the large spikes require relatively strong and rapidly rising stimuli would seem to be that with the greater increase in stimulus strength per unit time, the

chance for "grouped action" becomes greater. The number of elements recruited per unit time increases (see Discussion p. 66). Within limits it is possible to compensate for decreased rate of rise by increasing current strength, probably due to the increased frequency caused by such stimuli, as shown in fig. 9, record *a*. But there seems to be an upper limit for this "compensability".

Some experiments with micro-electrode leads in the muscle itself (m. tib. ant.) and the stimulating electrodes on the proximal stump of the cut sciatic are of particular interest in this

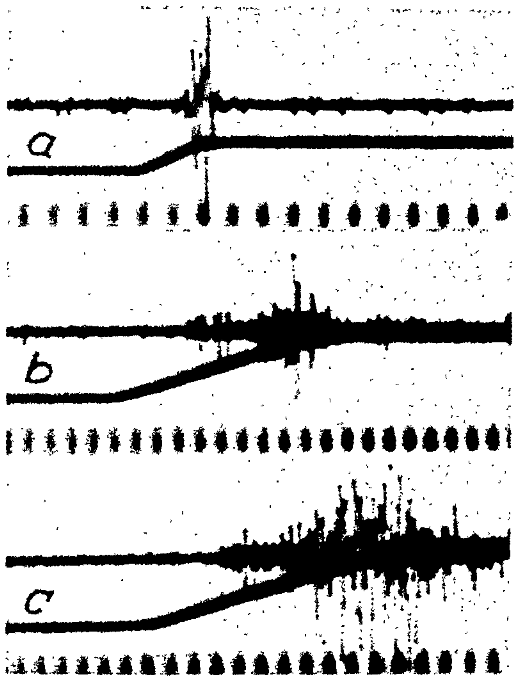


Fig. 19. Response to linearly rising current recorded with micro-electrode in the tib. ant. muscle. Time in 20 msec. See text. Size of largest spike in a: 500 μV.

connexion. The isolation obtained with such electrodes is by no means perfect, as pointed out by BERNHARD, GRANIT and SKOGLUND (1942).

The difference between small and large spikes is well marked also with this technique of leading off spikes from the muscle. In fig. 19, record *a*, a relatively high gradient of stimulation elicits large synchronized spikes. Record *b*, however, shows that, just as in the motor root, the slowly rising stimulus first elicits small spikes. But when it has risen sufficiently some large spikes also appear. In record *c* the gradient is the same as in record *b*, but the

current has been driven still somewhat higher. This leads to an iterative discharge of large spikes.

Most of the experiments carried out with leads in the muscle were finished at a time when the stimulator used rose exponentially, as in fig. 20, taken with a preliminary model of the stimulating device described above. The form of the current did not affect the principal findings, as is also well illustrated by the same figure. In the earlier experiments stimulus form was not recorded.

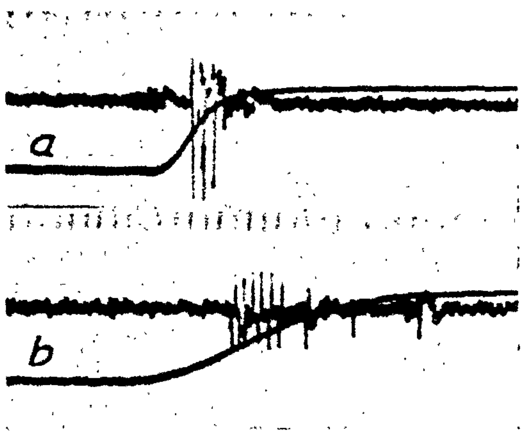


Fig. 20. Micro-electrode in tib. ant. muscle. Stimulation with exponentially rising currents. Time in 20 msec. See text.

Apart from the difference between large and small muscle spikes, the most important finding in these experiments was that the form of the accommodation curve was determined by the size of the spikes used as index for the threshold effect. As current form was not recorded it was necessary to use the appearance of a spike of a definite type as index for the threshold effect and plot current strength for this effect against fixed R $\times$ C values on the abscissa. Subject to the reservations made against this technique in the Historical section, the result was that the accommodation curves for large spikes rose more steeply than those taken with small spikes as index. This result is illustrated in fig. 21.

Also in a single experiment with the muscle contraction as index the same phenomenon turned up in the form that a certain gradient, with the threshold as index, elicited a contraction at twice rheobasic strength, whereas with a contraction corresponding to twice this amplitude as the constant index, three times the rheobase was required in order to make the same gradient effective.

This fact that the slope of the accommodation curve is a function of the size of the spike chosen as index, first seen with the muscle spike as index, is easily understood in the light of the results obtained with whole nerve, described above. These experiments clearly demonstrated that in order to obtain large synchronized spikes with slowly rising stimuli it was necessary to increase current strength far above the threshold. This, of course, means

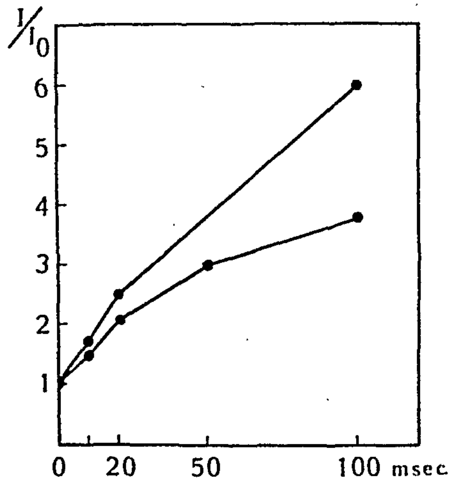


Fig. 21. Accommodation curves taken with muscle spike (recorded with microelectrode) as index. Upper curve: large spike as index. Lower curve: small spike as index. Abscissae: time constant of exponential rise (R × C).

that the slope of an accommodation curve determined with a large spike as index would increase in the same proportion. For this result to appear it is apparently immaterial whether muscle spikes or nerve action potentials are used as index and whether the curves are plotted in the manner used in this paper or as in some previous contributions to the subject of accommodation.

The results also serve to emphasize the unreliability of methods of measuring accommodation with slowly rising currents in which the index is a "constant" muscle contraction. Not only is the "constant" variable but the curve obtained is also a function of the index used. As pointed out by BERNHARD, GRANIT and SKOGLUND (1942) there is also the danger of changing over from one sort of index to another, at times without being aware of this change, which leads to the plotting of the gradually varying index against rising time rather than to a plot of strength of current against the same abscissa. It is true that the strict criterion for a reliable index in measuring the ordinates of the accommodation curves,

as applied in this paper, actually has led to a type of curve seen before — but only with frog nerve — namely, an initial straight portion curving down to a horizontal level when the stimuli rise more slowly. This does not necessarily mean that those who have previously published curves of the same general form also have plotted the same type of effect. It is, on the contrary, probable that they have plotted nothing but the gradual change of an initially constant index into one that alters character as the gradients of the stimuli decrease. The form of the later phase of the accommodation curve must actually be said to have been

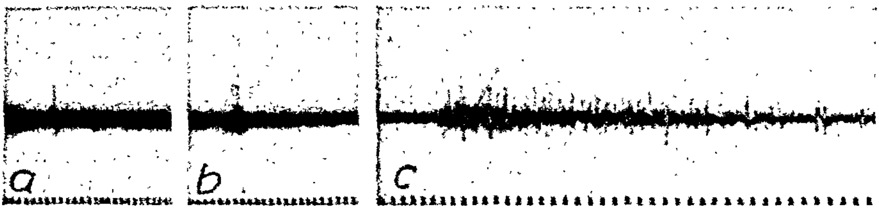


Fig. 22. Records taken with micro-electrode in tib. ant. muscle. Full description in text. Time in 20 msec. Size of small spike 200 μV; large spike 700 μV.

unknown owing to the fact that even those (see Historical section) who have been aware of the increasing repetitiousness with slowly rising stimuli have not taken any steps to ensure that the index throughout the measurements has remained constant.

Above it was suggested that the large spikes, seen particularly with suprathreshold stimuli of higher gradients, were due to grouped action of several fibres. Sometimes the records also show how spikes are grouped together with great regularity of frequency and height. In fig. 22 *a* there is first a small spike elicited at relative strength 8 and time-constant 10 msec. In record *b* stimulus strength has been increased to 10. There is then a discharge consisting of repetitive small spikes and a large one. These spike sizes were then kept up through the whole experiment. In record *c* they are shown for a slower gradient (100 msec.) and increased strength (strength 15). In this experiment the constancy of the grouped pattern was very high.

It is, of course, possible that grouped action is favoured by an abnormal state of nerve. But this tendency to grouping of the elements into constant spike patterns is also seen in experiments in which everything points to the nerve being normal. Thus, for instance, the seemingly isolated spikes from a root filament in

figs. 8, 9 and 10 are given by a *number* of fibres. This is shown by the fact that they tend to vary somewhat in size. More important still is that with brief rising times a small alteration of current strength may lead to a variation of spike height (see fig. 23). But there is a marked tendency towards formation of certain size patterns recurring with remarkable regularity despite variations in the mode of stimulation (figs. 8, 9 and 10).

Another example of grouped action in motor fibres are the impulses set up by the cut end of a nerve, studied by ADRIAN (1930). In fig. 24 *b* such discharges are recorded as spikes of relatively

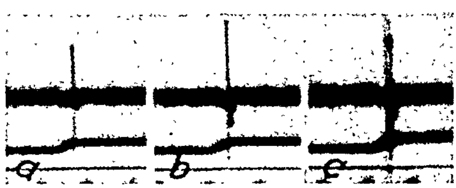


Fig. 23. Same experiment as in fig. 8. Leads on root filament. Constant gradient of stimulation. a, strength 28 microamp.; b, 32 microamp.; c, 34 microamp. Size of spike increases with increasing current strength showing that several elements are combined. Time in 20 msec.

constant size in the muscle. At the moment marking stimulation these are stopped by an anodal block. An interruption of the block leads to anodal opening discharge. Afterwards the spontaneous activity reappears and it is interesting to see how the spike height of the recurring discharge gradually reaches its original size. In fig. 24 *a*, a partial anodal block merely inhibits some of the elements contributing to the first spike after the block. This spike is here very thin on account of the removal of a fraction of its supporting elements.

5. Constant Current. Repetitiousness. Adaptation.

SOLANDT (1936 *a*) and KATZ (1936) showed that a decreased slope of the accommodation curve ran parallel with a greater tendency to repetitiousness in the nerves of frogs. Decalcification was the best method for producing a flatter accommodation curve. Systematic experiments on the effect of the external milieu have not been on the program of this work. In general, however, it may be stated that the correlation between the slope of the accommodation curve and repetitiousness has been verified whenever a chance has occurred to verify it. Asphyxia has quite often been seen to

favour repetitiousness (cf. LEHMANN, 1937) and then a flatter accommodation curve has been found. In the normal experiments, however, in which the variations in slope have not been considerable (cf. table 1, p. 37), the tendency to fire repetitively has also been much the same in the different cases.

Despite the parallelism between slope of accommodation curve and repetitiousness it would be a mistake to regard a repetitive discharge merely as the direct consequence of deficient accommo-

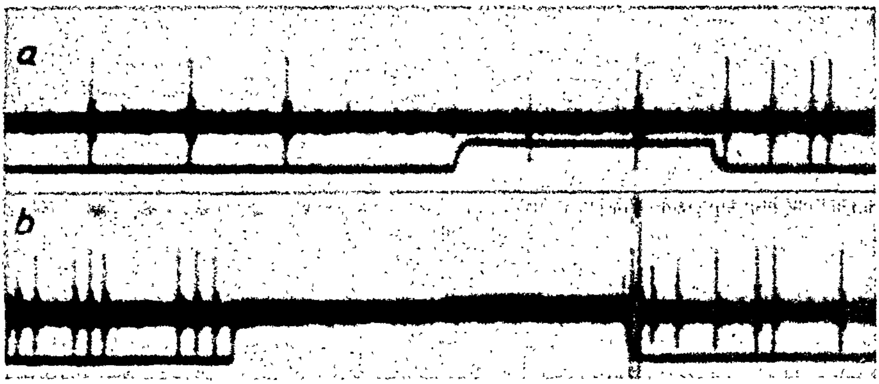


Fig. 24. Impulses set up by the cut end of the sciatic nerve, records being taken with micro-electrode in tib. ant. muscle. Stimulus marked on figure represents anodal block on nerve. a, relative strength 10 only causes partial block; b, relative strength 18 causes complete block and in addition opening discharge. See text. Time in 20 msec.

dation, as done, for instance, by KATZ (1936). Once the autorhythmic mechanism is released, and breakdown of accommodation has occurred, this mechanism turns up with interesting properties of its own which cannot be forced together under the simple heading of accommodation. Some of the evidence for this conclusion will now be set forth.

What is, to begin with, the strength of current necessary for prolonged repetitiousness? I do not now mean the brief iterative discharge following upon any suprathreshold stimulus. On account of physiological variations the threshold for prolonged repetitiousness may be difficult to determine with great precision. But as a rule this threshold in the motor roots is at a value of 3—4 times the rheobase (5 experiments). In some experiments (2) repetitiousness required 5 times the rheobase. The presence of a threshold for this phenomenon explains breakdown of accommodation: it means that at this level the rate of rise of the stimulus looses its

significance. The horizontal portion of the accommodation curves corresponds to this level although a successive departure from the initial linearly rising phase of the curves precedes horizontality.

In fig. 25 a repetitive discharge to a constant current has been led off from a motor root filament. Record *a* shows stimulation

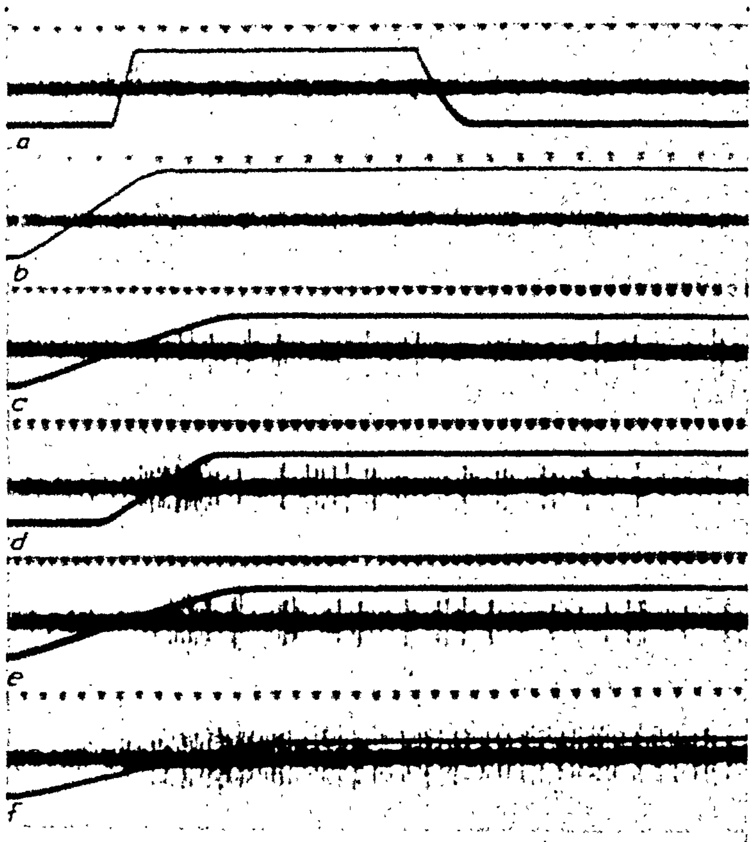


Fig. 25. Leads on motor root filament. Stimulation of sciatic nerve. Illustrating repetition during plateau portion of stimulus at different current strengths which in terms of multiples of rheobasic strength are: a: 1.7. b: 3.0. c: 4.0. d and e: 5.0. f: 6.2. See text. Time in 20 msec.

with a strength of 1.7 times the rheobase leading to two spikes during the rising phase of the stimulus. Record *b* corresponds to strength 3 which still is below the threshold for repetitiousness. Record *c*, corresponding to a strength of 4 times the rheobase, shows a repetitive discharge of slow frequency. If now the strength of the stimulus be increased to 5, the frequency of the discharge increases, as illustrated in records *d* and *e*. The differences in the rate of rise of the stimulus can be seen to affect the frequency of the discharge merely during the rising phase, as already pointed

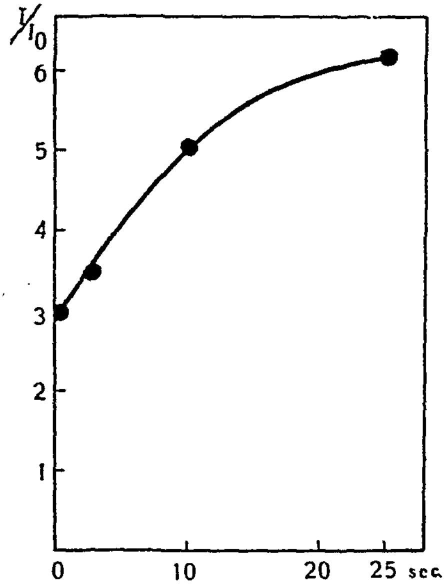


Fig. 26. Duration of repetitive firing in sec. as function of stimulus strength in multiples of rheobasic strength.

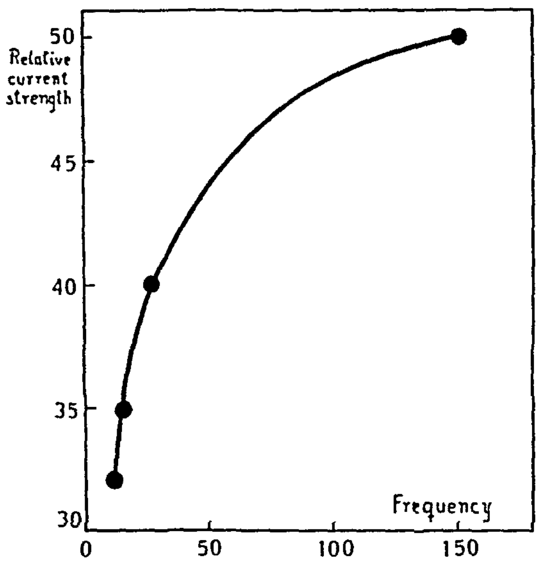


Fig. 27. Frequency of repetitive discharge as function of relative current strength.

out above (p. 32), but to be without influence on the prolonged repetition. In the last record (*e*) excitation is 6.2 times the rheobase.

Repetitive firing is found to continue for a considerable time at a decreasing rate. There is thus *adaptation* to the stimulus. As a consequence of the adaptive processes the nerve sooner or later ceases to respond. How long it is capable of keeping up its discharge

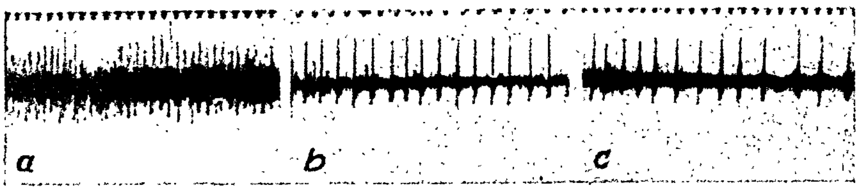


Fig. 28. Stimulation of sciatic nerve with constant current. Micro-electrode records from tib. ant. muscle. Relative current strength and spike frequency per sec. in the different records are respectively: a, 20 and 90, b, 15 and 50, c, 10 and 35. Time in 20 msec.

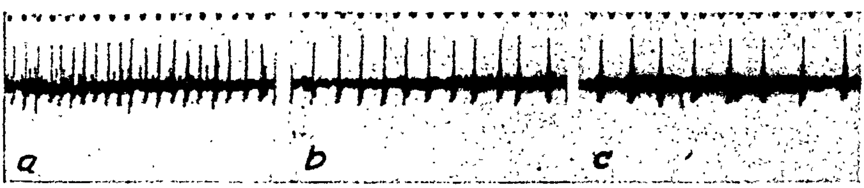


Fig. 29. As in fig. 28, but showing spike frequency as function of duration of stimulation; a, immediately 60 per sec., b, after 2 sec. 35 per sec. and c, after 5 sec. 25 per sec. Time in 20 msec.

depends among other factors upon the strength of the stimulus. This relation is illustrated in the diagram of fig. 26, in which duration of repetitiousness is plotted against strength of stimulus. These durations are of an order of magnitude which would seem to exclude any simple relation to the time constant of accommodation such as the one claimed by Katz (1936) for frog nerve. The inverse value of the slope is about 40 in the experiment from which the diagram is taken.

Of great interest is also the *frequency* of these discharges. It is surprisingly low at the threshold and increases when the strength of the stimulus increases. This fact is illustrated in fig. 27 from an experiment in which a large spike of constant size was well isolated with microelectrodes in the muscle. Up to 150 per sec. it was easily followed, but, when stimulus strength was further increased, it disappeared in the large number of action

potentials from surrounding elements. But it could be seen still to increase in frequency above the limit within which accurate counting was possible. The relation between stimulus strength and frequency is illustrated in fig. 27.

In fig. 28, there are some records from another experiment with micro-electrodes in the muscle, in which the regular increase in frequency with stimulus strength is well illustrated.

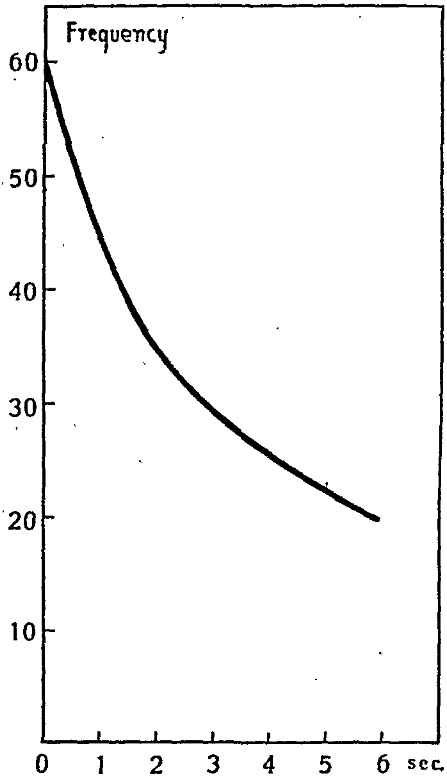


Fig. 30. Adaptation in motor nerve. Spike frequency as function of duration of stimulation with constant current.

The spikes with their relatively constant amplitude are typical for "grouped action" in motor nerve. Fig. 29 shows the gradually decreasing frequency after different intervals — 0, 2 and 5 sec. — from the moment of stimulation. The diagram of fig. 30 illustrates the gradual drop in frequency in this experiment as a function of duration of stimulation.

All the facts presented in this section might equally well have been obtained with a sense-organ developing generator potential, and serve to illustrate the applicability of this model to problems raised by the electrophysiology of the sense organs.

II. Sensory Nerves.

1. General Results. Limits of Variation.

Use of the index described on the previous pages means that the effect of slowly rising currents can be studied on sensory nerves just as easily as on motor nerves. Some results of such experiments will now be reported. The general arrangement has been analogous to the one used for motor nerves, that is, stimulation of the sciatic nerve and leads on the sensory root instead of on the motor root. As a rule both experiments were performed alternately on the same animal so that the stimulating electrodes could be left untouched and the amplifier merely be switched over to leads on the root wanted. In such experiments there were excellent opportunities for a quantitative comparison of the behaviour of sensory and motor nerves, as possible changes in the internal and external milieu would affect both sets of fibres simultaneously. The sensory fibres studied by this method would be low-threshold ones of large diameter and thus probably come from the muscle proprioceptives (SHERRINGTON, 1894). Correction for conduction velocity has been carried out on a basis of 80 m. per sec.

In 5 experiments the thresholds for these fibres at the rheobase have been compared with the thresholds for the motor fibres of highest sensitivity. The average value for the rheobase of the sensory fibres is 24 % below the average for the motor fibres. This value is relatively close to ERLANGER and BLAIR's (1938) corresponding figure for frogs (20 %).

The records of fig. 31 reproduce some typical results of stimulation of sensory fibres when the leads are on the roots.

The broad baseline, compared with earlier figures referring to motor nerves, is a consequence of spontaneous activity which was considerable in these experiments. It did not seem unreasonable to assume in the first instance that this activity came from sense organs innervated by branches *above* the severed end of the sciatic. But as the spontaneous activity was greatly augmented after section of all the branches, belonging to the root recorded from, I concluded that they were set up by the cut surfaces, as described by ADRIAN (1930). They also diminished greatly during the experiment, after some time to such an extent, that a relatively high degree of accuracy in the reading-off of the latent periods

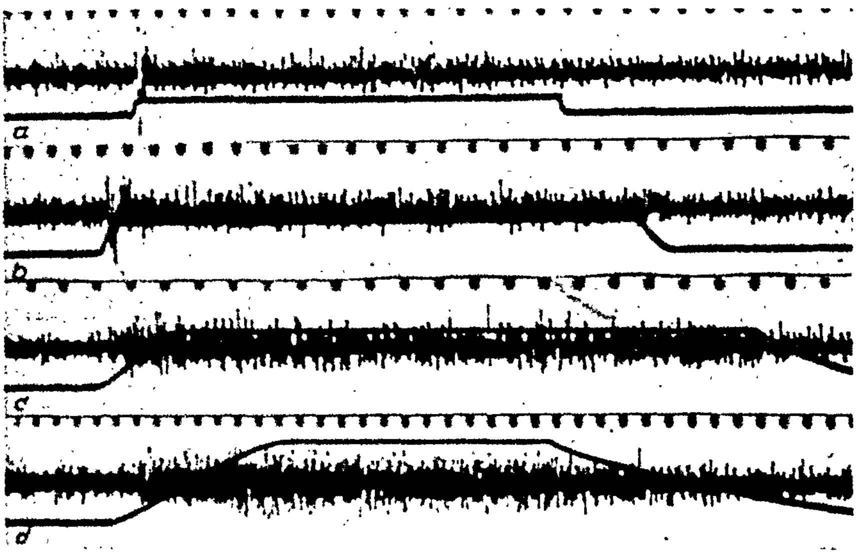


Fig. 31. Leads on sensory root. Stimulation of sciatic nerve. Time in 20 msec. Size of large spike in a: 150 μ V. For each record rising time, final current strength in multiples of rheobasic strength, latent period and strength at latent period are as follows:

a: 5 msec. 1.2; 5 msec. 1.2.
b: 10 msec. 1.5; 8 msec. 1.2.
c: 40 msec. 3.5; 15 msec. 1.3.
d: 140 msec. 5.0; 35 msec. 1.3.

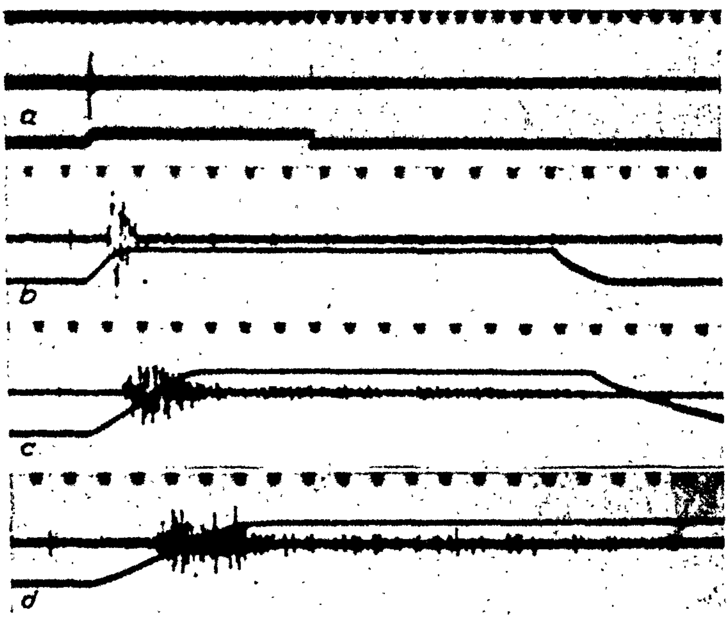


Fig. 32. Leads on motor root. Stimulation of sciatic nerve. Showing strength necessary for repetition to be compared with fig. 31 for sensory nerve. a, rheobase, b, 2.5, c, 4.0 and d, 5 times rheobase. Time in 20 msec.

of the spikes caused by stimulation could be reached. The error caused by the spontaneous activity represents a slight lengthening of the latent period on account of the difficulty in tracing the first reponse against the background of injury discharge.

In the records of fig. 31 it can easily be seen that there is a relatively moderate lengthening of the latencies with decreasing gradients of stimulation. This, of course, means a relatively slight increase of stimulus strength relative to the rheobase.

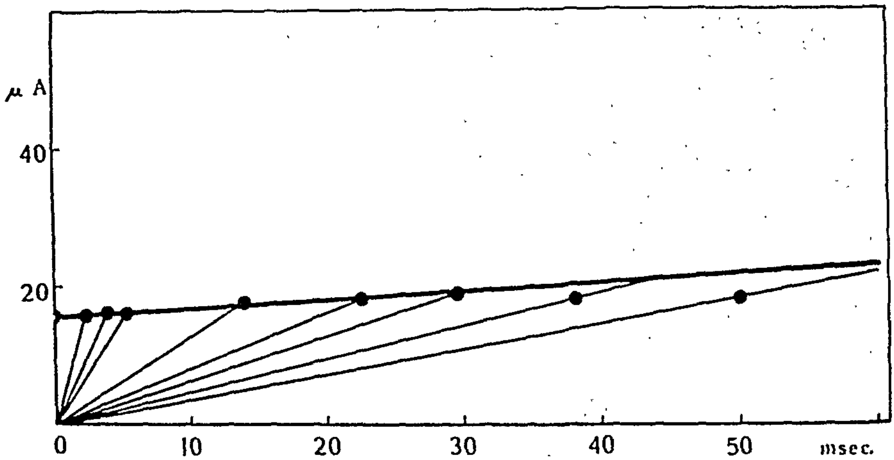


Fig. 33. Accommodation curve for sensory fibres. See text.

In fig. 33 is found the typical result of plotting the latent periods on the rising stimuli in the same manner as for motor nerves. The values for the brief rising times have been obtained by the procedure of threshold testing described in detail for motor nerves (p. 38). I have drawn a straight line through the 7 first points in the diagram, and this clearly represents an accommodation curve of very moderate slope. If these values are recalculated in terms of multiples of rheobasic strength the inverse value of the slope of this curve is 160.

We can see that at the two slowest rising times in fig. 33 the latent periods have not increased above the just preceding value. This is emphasized by the curve continued as a straight line beyond this point. The last experimental readings are far below it. Thus breakdown of accommodation sets in at a current strength which on an average is only 1.5 times the rheobase. In fig. 33 the point in the diagram representing the slowest gradient is actually somewhat below the preceding value. But with as slow gradients as those at the end of the curve of this figure the results tend to

vary and it is not worth while to expand the observations further in this direction.

Fig. 34 demonstrates a typical result from an experiment in which both the motor and sensory roots were in turn connected to

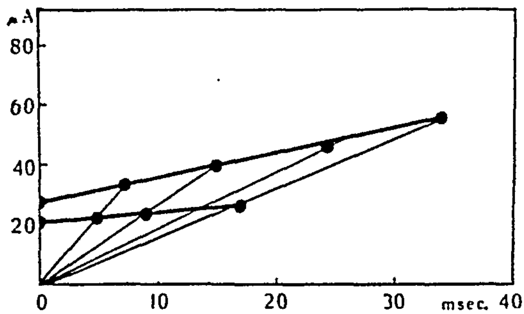


Fig. 34. Accommodation curves for sensory and motor fibres compared. Upper curve: motor, lower curve: sensory. Ordinates: relative current strengths. See text.

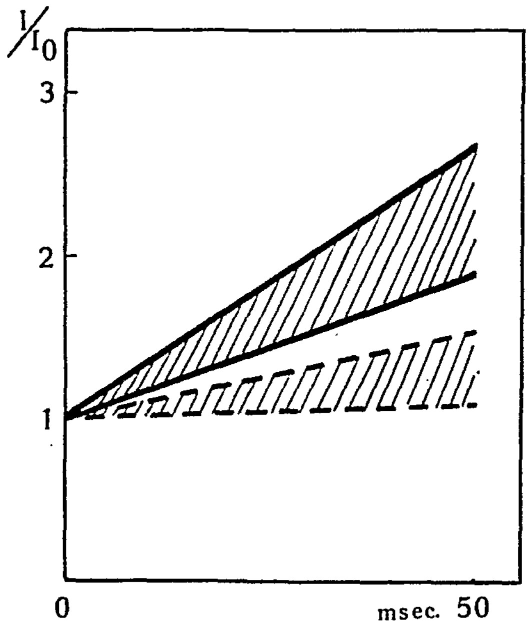


Fig. 35. Limits of variation of slopes of motor and sensory accommodation curves. Lines drawn in full: motor. Broken lines: sensory (including *n. saphenous*).

the amplifier so that the results can be directly compared. The latent periods for both motor and sensory nerves are plotted on the rising stimuli against stimulus strength, as directly read off from the ammeter. Both the difference in threshold and slope of the accommodation curves can accordingly be seen on the same

diagram. But a strictly quantitative comparison requires replotting of the same data in terms of multiples of rheobasic strength (see the average result for motor and sensory nerves, fig. 35).

The examples selected show the very marked difference between the accommodation curves of motor and sensory nerves. This difference as well as the extreme limits of variation are well illustrated by Table 1 in which the inverse value of the slope of the initial linearly rising portion of the curve — up to 50 msec. — has been tabulated for motor and sensory nerves.

The diagram of fig. 35 shows the range within which the slopes have varied, separately for motor and sensory nerves, and within rising times of 50 msec. The rest of the curve in both cases then gradually and somewhat irregularly passes over into a horizontal portion, as has been shown in connexion with a description of breakdown of accommodation for motor nerves and above (fig. 33) for a sensory nerve. In motor nerves this level corresponds to 3—4 times, in sensory nerves to 1.5 times the rheobase.

2. Some Special Results.

As indicated by the general course of the accommodation curve repetitiousness is considerable in sensory nerves. In fig. 31 there is repetitive firing, already in record *b*, at 1.5 times the rheobase, not only during the rising phase as in motor nerves but also during the time when the stimulus remains at plateau height. In the

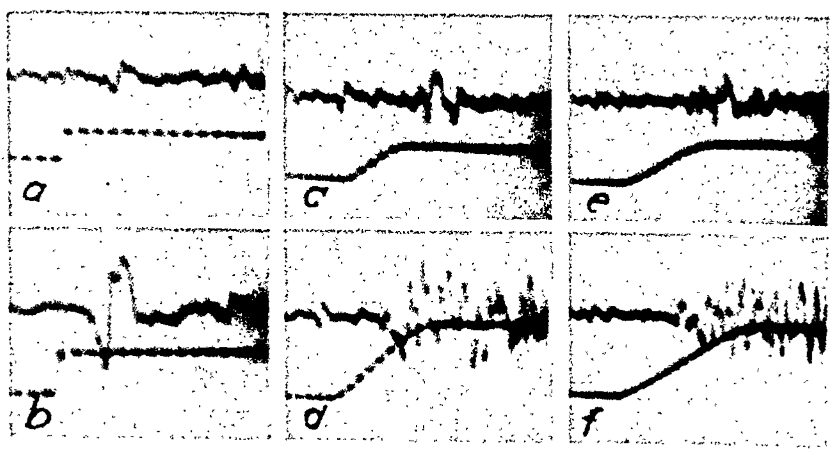


Fig. 36. Leads on sensory root. Stimulation of sciatic nerve. a and b, instantaneous current rise; c and d, very fast gradient; e and f, less steep gradient. Explanation in text. Time in msec. Size of spike in b: 75 μ V.

following records, *c* and *d*, corresponding to 3.5 and 5 times the rheobase the rhytmic activity is very heavy. In all the experiments with leads on sensory roots repetitive firing has regularly turned up at strengths corresponding to 1.5—2 times the rheobase.

For comparison I have inserted as fig. 32 a picture from the motor fibres of the sciatic in which repetitiousness turns up at a strength of 4 times the rheobase.

Adaptation to the constant current, described above (p. 52) for motor fibres, is seen also with sensory fibres. The duration

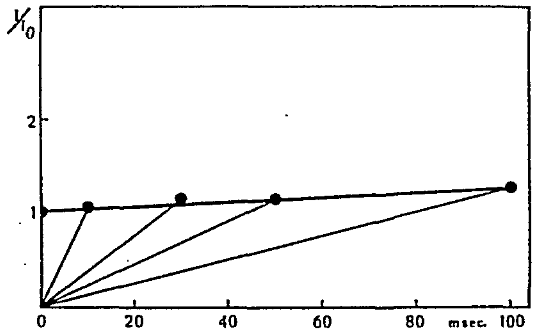


Fig. 37. Accommodation curve for saphenous nerve.

of the discharge, however, is definitely longer in sensory than in motor nerves for the same multiples of rheobasic strength. Systematic measurements of this difference have not been carried out.

In fig. 36 are found some pictures illustrating action potentials from the dorsal root filaments in response to stimuli of brief rising times. Record *a* is a threshold stimulus at the rheobase. Strength of current is 20 microamp. and the impulse follows after a latent period of 1.75 msec. In record *b* is seen a greater response to a strength of 30 microamp., following after a latency shortened to 0.50 msec.

In records *c* and *d* the stimulation gradients are identical; the former rises to 24 microamp., the latter to 52 microamp. Also in this case there is a just noticeable shortening of the latent period for the stronger stimulus amounting to about 1 msec. In records *e* and *f* the gradients have been diminished. A difference in latent period is here not visible, though the latter stimulus is stronger. The records *d* and *f*, for which stimulus strength is about 2.5 times the rheobase, show that the nerve has responded repetitively, not merely to the rising phase but also during the plateau level. These curves should be compared with those taken from the motor

nerve — see fig. 17 — which is silent during the plateau level of stimulation.

Two experiments have been carried out with the saphenous nerve. Leads were applied both to the corresponding dorsal root and to the nerve at a distance of a few centimeters from the stimulating electrodes. The pictures obtained were similar in

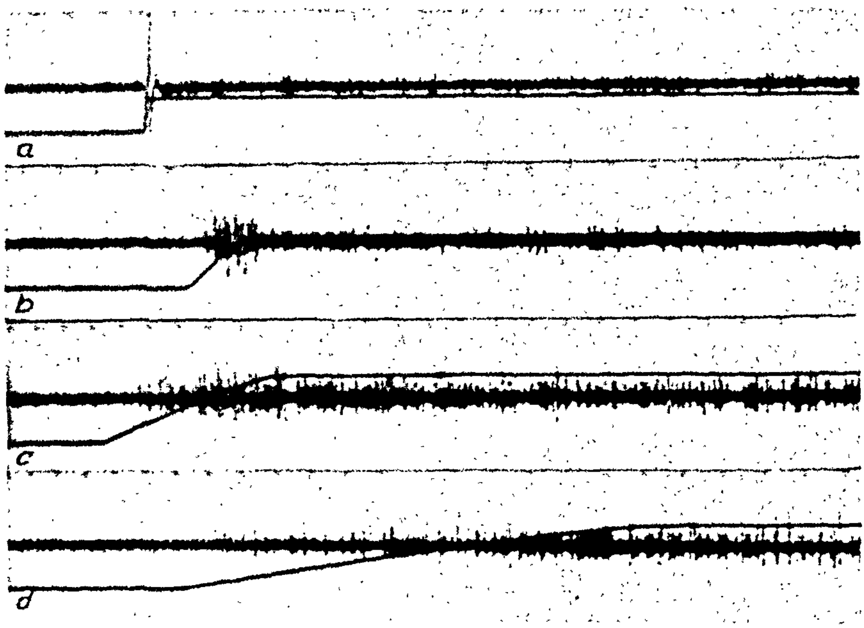


Fig. 38. Leads on saphenous nerve. Stimulation of same nerve with stimuli of different gradients. Time in 20 msec. For each record rising time, final current strength in multiples of rheobasic strength, latent period and strength at latent period are as follows:
a: instantaneous 1.1.
b: 30 msec. 2.0; 17 msec. 1.1.
c: 100 msec. 3.2; 35 msec. 1.1.
d: 250 msec. 3.2. The frequence of the discharge increases gradually and irregularly with latent period corresponding to current strength below 1.0.

both cases and these fibres, which according to HEINBECKER, O'LEARY and BISHOP (1933) are purely sensory and possess conduction velocities of about 60—80 m. per sec. for the A group, had very little accommodation. In the one experiment the inverse value of the slope was 270 (see fig. 37), in the other approaching an infinitely high value, the slope being practically horizontal.

In fig. 38 there are some records taken directly from the saphenous nerve. The latent period is very short and with slow gradients the impulses come irregularly at a strength of stimulation corre-

sponding to the region of threshold play around the rheobase. The rheobase is difficult to measure precisely, on account of the stimulus artefact, when the leads are so near the excited locus.

Record *a* in fig. 38 shows that the saphenous nerve responds with some iterated impulses just above the rheobase and that repetitive firing is well marked at a strength corresponding to twice the rheobase, record *b*. The rest of the curves have been obtained with stimuli 3.2 times the rheobase.

An important difference between sensory and motor nerves is also seen in the fact that the tendency towards synchronized or grouped discharges is far less evident with sensory than with motor fibres. It is seen only at the rheobase and with the fastest gradients (cf. fig. 31). As is shown in fig. 38 from the saphenous nerve, it is possible to raise the strength of the stimulus to values above the rheobase without obtaining spikes which are significantly larger than those seen at the threshold.

The typical picture with the motor nerve, as pointed out above, was a grouping of the potentials to large spikes of constant size. This characteristic picture was not seen at all or was less well marked with the sensory nerves.

Discussion.

1. The Accommodation Curves.

The use of linearly rising currents instead of exponential ones, advocated in the introduction, has greatly furthered the analysis of the phenomena noted in stimulation with slowly rising currents. Of particular importance has been that the linearly rising stimulus for any constant gradient could be driven up to any desirable height. My method of measuring accommodation has been based on this technique. It has been shown that stimulus strength at the latent period of the first spike, photographically determined in parallel with simultaneous recording of stimulus form, can be used as a measure of threshold stimulus strength for all gradients except the fastest ones. In this manner the accommodation curve can be conveniently determined with the same degree of accuracy for all kinds of nerves.

With this index the accommodation curve refers to fibres which have low threshold for the gradients used. (In this respect my method agrees with other methods of measuring accommodation.) I have assumed that the points plotted on the accommodation curve refer to the *same* fibres. This assumption presupposes that the accommodative properties of the fibres of somewhat smaller diameter do not differ significantly from those of the largest fibres. Assuming, however, that a group of thinner fibres with a rheobase of twice that of the low-threshold fibres would have very little accommodation, then it might be possible that — despite the threshold difference at the rheobase — these fibres would respond to very slow gradients before the other ones. As yet we know nothing of the accommodative properties of different fibre groups, and therefore a possibility like the one imagined cannot be excluded. But the results of the experiments with small strands of fibres from the motor root filaments, in which the potentials were of constant size through all gradients of stimulation, certainly suggest that in this case the same elements

or element responded throughout the series. This shows it to be very unlikely that the accommodation curves found in these experiments could be due to a shifting-over from, say, one type of element with much accommodation to another with little accommodation.

As regards the saphenous nerve we know that the measurements refer to a group of elements with conduction velocities from 80—60 m. per sec. (HEINBECKER, O'LEARY and BISHOP, 1933) throughout the whole accommodation curve. The following group of fibres with a conduction velocity of about 30—20 m. per sec. has already at the rheobase a threshold of 4.5—6 times the rheobase of the fast group. The increase in current strength necessary for eliciting activity with the slower gradients has nowhere been anywhere near this amount.

Correction for conduction velocity has been based on values somewhat below the top velocities. The reason for this has been that it has seemed improbable that the fastest fibres would be represented at the threshold in such numbers as to be capable of constituting the index spike alone. But a variation of some 10—20 m. upwards or downwards in this value at the slow gradients would also be of very little significance compared with other sources of error. For the fast gradients the latent periods have not been the means of measuring the threshold effects. They were there measured directly.

The accommodation curve of the motor nerves has been discussed in relation to some earlier results above on pp. 35—38 and 46 but some points still deserve to be mentioned.

Typical for the mammalian motor nerve is an accommodation curve with a relatively steep initial slope signifying a considerable degree of accommodation to slowly rising currents. The mammalian nerve is clearly lacking an absolute *pente limite*. Instead of the nerve refusing to respond to gradients below a certain limit its mode of reaction altogether changes character: accommodation breaks down and it begins to discharge iteratively in a manner which is determined by current strength and not by current gradient. This release of the autorhythmic mechanism takes place at a strength of current some 3—4 times the rheobase.

The result suggests some caution in accepting the existence of the *pente limite* in excised frog nerve (cf. Historical section p. 14). It is probably a pathological phenomenon. Some results

by LIESSE (1938 b) are in favour of this conclusion. On frog nerve *in situ* he showed that interruption of the circulation led to increased accommodation. But that accommodation in circulated nerve should be negligeable, as held by PARRACK (1940), is clearly erroneous if accommodation be defined as in this paper.

In experiments on man EBBECKE (1924) came to the conclusion that suprathreshold stimuli often led to iterated activity in both motor and sensory nerve. Recently ROSENBLUETH (1941 b) has recorded from mammalian nerve *in situ* and found repetitive discharges to be the typical response to strong stimuli.

From this point of view stimulation with slowly rising currents is of especial interest. Not only does it correlate the threshold of repetitiousness with accommodation but it also separates the brief rhythmic responses to the rising stimulus from the prolonged repetitiousness during the horizontal level of stimulation. The prolonged repetitiousness presupposes that the accommodative mechanism somehow has broken down and it is probably a phenomenon *sui generis*. In earlier experiments with the muscle contraction as index slowly rising stimuli have been found to be accompanied by repetition (see Historical section p. 18) but it has not been possible to state which phase of the current has been responsible for this effect.

By following the effects on the screen of the cathode ray and carefully adjusting stimulus strength it has actually been possible to elicit single spikes also with slowly rising stimuli. The repetitive discharge therefore indicates suprathreshold stimulation. Still, the general impression of the effect of slower gradients is that nerve stimulated in this manner actually is prone to discharge iteratively, as if there were a greater instability in the excitatory process with such stimuli. ROSENBLUETH's (1941 a) recent experiment on excitability changes at the cathode and anode of mammalian nerves responding to galvanic stimulation indicates that this actually is the case.

Considering that repetitiousness during the plateau portion of the stimulus at certain multiples of rheobasic strength is independent of the form of the rising phase of the stimulus, LAPICQUE (1937 b) would seem to be right in refusing to accept the accommodation curves derived from work on threshold contractions, inasmuch as they have been complicated by repetitive activity at the slower gradients. LAPICQUE has pointed out that the autorhythmic mechanism represents a specific form of activ-

ity. FABRE (1936 a) has also emphasized that slowly rising stimuli may activate a physico-chemical system with other properties than the ones tested by the prevalent shock technique. In the last section on nerve as model sense organ, I shall return to the properties of the autorhythmic mechanism.

The differences in the accommodation curves of motor and sensory nerves, as we have seen, are well marked and the slope of the initial portion or its inverse value provides one with an accurate expression for this difference. From this would follow that accommodation in the saphenous nerve would be less marked than in the sciatic fibres. The experiments with the saphenous are, however, too few to justify a definite conclusion.

Another difference between motor and sensory nerves is the lower threshold of repetitiousness of the latter, seen also by ERLANGER and BLAIR (1938) with excised frog nerve. They have suggested as an explanation that the sensory nerves have a looser membrane than the motor ones. ERLANGER and BLAIR have also drawn attention to a number of facts which support this hypothesis.

Among other differences between motor and sensory nerves, found in this work, I would like to emphasize those referring to duration of repetitiousness and to grouped action, to be discussed separately below.

To sum up: the differences between motor and sensory mammalian nerves are according to my results:

1. The rheobase of the sensory fibres is on an average 24 % below that of the motor nerves.

2. The inverse values of the slope of the rectilinear initial portion of the accommodation curve varies for motor nerves between 30—60, signifying a considerable capacity of these nerves to accommodate — in the sense of HILL. The corresponding values for sensory nerves vary from 90 to infinity, signifying that they accommodate far less or not at all.

3. For motor nerves there is breakdown of accommodation at 3—4 times the rheobase. At this strength is also the threshold for prolonged repetition. The corresponding value and threshold for prolonged repetitiousness for sensory nerves is at 1.5—2 times the rheobase.

4. For equivalent multiples of rheobasic strength the duration of repetitiousness of the sensory nerves exceeds that of the motor nerves.

5. The severed motor nerves have little or no spontaneous activity, the sensory nerves very pronounced spontaneous activity.

6. The motor nerves show a tendency to grouped action leading to composite spikes of constant height, single or repetitive, and sometimes of considerable size. This is seldom or not at all seen with sensory nerves.

These differences suggest that, whatever the nature of the autorhythmic mechanism of nerve, this mechanism is better developed and possesses a lower threshold in sensory than in motor nerves.

Finally it should be pointed out that the comparison refers to A fibres.

2. Grouped Action.

The main question with regard to grouped action (see above p. 43) in motor nerves is whether this phenomenon can be explained merely by the greater probability of coincidence of spikes of different fibres or whether grouping takes place as a consequence of some physiological or anatomical arrangement favouring it. Alternatively all three factors, statistical chance, anatomical grouping, physiological interaction, combine to the same end effect.

It would seem as if no explanation of this phenomenon could be satisfactory unless it also can explain that grouped action is present in practically all records from motor nerves and absent or less definite in sensory nerves. The most obvious anatomical difference between motor and sensory nerves is the greater range of variation in fibre size of the latter. But it is questionable if this difference can play any rôle as long as one is studying threshold phenomena, as is done during the rising phase of stimulation, when grouped action is so marked. However, during this phase the probability for simultaneous activation of fibres of identical properties as to threshold and conduction velocity is greatest. In this sense a statistical chance may play a rôle and perhaps explain the fact that grouped action is so well marked with the relatively faster gradients. But there again the difference between motor and sensory nerves is present, and some additional factor would be necessary to explain it. From this point of view it is of particular importance that grouped activity

in motor nerves by no means is restricted to steeply rising stimuli, though favoured by them. For this reason it is necessary to conclude that some other factor than probability is operating in determining grouped action.

Already in 1930 ADRIAN explained the synchronized activity of spontaneously active excised mammalian nerves by assuming that an active fibre can influence another and cause a slight momentary increase in the stimulus of other fibres owing to the action current which it produces.

JASPER and MONNIER (1938), KATZ and SCHMITT (1940) and ARVANITAKI (1940) have shown that in evertebrate nerve the action potential of one fibre influences another, KATZ and SCHMITT demonstrating how an asynchronous discharge of two fibres can be synchronized by interaction. Recently ROSENBLUETH (1941 c) has shown that by increasing the excitability of a myelinated axon (cat) through local stimulation with direct current the action potentials carried by some fibres can stimulate adjacent fibres.

Thus there is evidence for a physiological mechanism of synchronization of activity. My results are certainly best explained by assuming them to be caused by a similar mechanism.

This makes the difference between motor and sensory nerves with respect to grouped action still more interesting. At the moment I can only suggest that the different accommodative properties of motor and sensory nerves is the common cause behind this striking difference with regard to grouped action. An efficient accommodation may serve to compress the time limit within which impulses are born. If this be so the mechanism of interaction may well prove to be of great importance in the normal reflex activity of the motor nerve. Actually records of so-called motor units, say, for instance in stretch reflexes indicate that grouped activity is the normal form of discharge of the motor fibres.

3. Nerve as Model Sense organ. Repetitiousness.

As pointed out by ADRIAN and ZOTTERMAN (1926) the principle upon which the sense organs are built up seems to be that steady excitation yields a rhythmic response and that the rate of discharge in this response rises with increasing excitation. Anodal and cathodal tetani, noted from the beginning of electrophysiology, offered the nearest analogy to the kind of mechanism

that seemed to dominate sense organs. But unfortunately the rhythmic mechanisms of nerve have received very little attention, and in the background there always remained the question as to how accommodation and iterative firing could co-exist. For this reason BERNHARD, GRANIT and SKOGLUND (1942) emphasized the need for a quantitative analysis of the problem "nerve as model sense organ" in which accommodation and repetitiousness should receive especial attention. This paper supplies the information wanted. The nearest approach to a similar study is the work of ERLANGER and BLAIR (1936) with frog nerve in which a constant current was used and accommodation accordingly measured in a manner very different from that found in earlier and later papers with the exception of that of PARRACK (1940). This work has been reviewed in the Historical section.

For several reasons the model sense organ seemed to be best imitated by slowly rising stimuli and this view we have now found justified by the ease and precision with which quantitative data about accommodation and repetitiousness have been simultaneously obtained. The results show that the model works. A review of its properties would in the first instance emphasize the following points.

In both motor and sensory nerves the effectiveness of the accommodative mechanism is limited to a certain region of stimulus strength. This in sensory nerves is so narrow — maximally twice the rheobase but generally still narrower or even absent — that a force capable of setting up a generator potential in the sense organ should be able to overcome the accommodative resistance without demanding improbable quantities of energy. The sensory nerves are thus particularly well adapted to the task of responding to generator potentials like those we know from the work on the retina.

When the stimulus rises slowly it is nevertheless effective at the threshold for breakdown of accommodation, which is also the threshold for the release of the autorhythmic mechanism of the nerve. This mechanism is present in both motor and sensory nerves and qualitatively similar in both. But it is released far more easily in sensory than in motor nerves. By decreasing the rate of rise of the stimulus one can postpone the moment of release of the rhythmic discharge just as in the retina a decreased rate of rise of the generator potential runs parallel with a lengthened latent period.

The autorhythmic mechanism of the nerve has properties which strikingly agree with what is known about the properties of the sense organs. Thus the frequency of the rhythmic discharge increases with stimulus strength (see fig. 27 p. 51). This has also been pointed out by FESSARD (1936) on the basis of experiments on frog nerve. The fact is of particular interest not only because such a state of affairs is a theoretical requirement but also because it points to the genuineness of the autorhythmic mechanism. Thus, for instance, KATZ (1936) has taken the view that the rhythmic response merely is a consequence of delayed accommodation. If this were so the nerve should discharge with a frequency determined by its refractory period. Instead, what we find, when accommodation ceases to operate, is the release of a genuine rhythmic mechanism with properties particularly adapted for making it serve as the vehicle of propagation of the sensory message. Most interesting is also that the nerve adapts to the constant stimulus (fig. 30 p. 53) in a manner highly reminiscent of adaptation in sense organs.

The linearly rising current is probably the best approximation to the natural mode of stimulation of a sense organ with a generator potential such as the retina. How it stimulates, whether merely by depolarizing the nerve or by in addition setting up a local potential, we do not know.

Summary.

1. For the analysis of the response of mammalian nerves to slowly rising currents a new technique has been developed. It is based on the use of a stimulating device (fig. 6 p. 25) delivering linearly increasing currents in which gradient and current strength are independent variables. Thus it has been possible to drive the current up to any desirable height for any constant gradient. The stimulator is connected to the one beam of a double cathode ray oscillograph recording stimulus form, and, if wanted, also stimulus strength though, as a rule, stimulus strength has been measured separately with an ammeter. Onto the other beam of the cathode ray is connected a condenser-coupled amplifier recording the action potentials of the nerve stimulated.

Experiments have been carried out on decerebrate cats in which the sciatic nerve has been stimulated and the response recorded from either the motor or the sensory roots or from the root filaments.

2. By means of this technique and photographic recording it has been possible to measure the moment at which the nerve responds, in relation to the strength to which the linearly rising current has risen at this moment, identical with the latent period of the first impulse. It has been shown that stimulus strength at the latent period of the first spike of the response is an accurate measure of threshold strength for all gradients except the fastest ones. For these it has been necessary to use the classical method of measuring threshold strength directly.

In this manner curves relating current strength to current duration for variable current gradients, so-called accommodation curves, can be accurately plotted for both motor and sensory nerves.

3. Measurement of accommodation by this method leads to uniform and reproducible results which is not the case when a muscle contraction or the size of the action potential is used as index for measuring accommodation. This conclusion is based

on a comparative study of the reliability of the index in measuring accommodation.

4. Typical for the mammalian motor nerve is an accommodation curve with a relatively steep initial slope signifying a considerable degree of accommodation to slowly rising linear currents.

The inverse value of the slope of the initial part of the accommodation curve varies between 30—60. As a rule the initial slope is linear already from the beginning, sometimes, however, successively diminishing.

The rectilinear initial phase of the accommodation curve is succeeded by a slowly diminishing slope passing over into a horizontal phase corresponding to about 3—4 times rheobasic strength of the stimulus.

Thus the mammalian motor nerve is lacking an absolute *pente limite.* Instead of the nerve refusing to respond to the slower gradients of stimulation below a certain limit its mode of reaction altogether changes character, accommodation breaks down, and the nerve begins to discharge iteratively in a manner which is determined by current strength and not by current gradient.

There is thus a release of an autorhythmic mechanism at a current strength of on an average 3—4 times the rheobase.

5. The accommodation curve of the sensory fibres is very much flatter, though of the same general type. The inverse value of the slope of the initial linear portion is between 90—200. Still higher values have been reached with the saphenous nerve. Thus the sensory nerve accommodates far less than the motor nerve, sometimes not at all. But as a rule breakdown of accommodation takes place at a value of about 1.5—2 times rheobasic strength. As in motor nerve, this marks the threshold for prolonged repetitive discharges. Thus the threshold for prolonged repetitiousness is lower in sensory than in motor nerves.

6. Further differences between mammalian motor and sensory nerves are:

The rheobase of the sensory nerves is on an average 24 % below that of the motor fibres.

For equivalent multiples of rheobasic strength the duration of the repetitive response of the sensory nerves exceeds that of the motor nerves.

The severed motor nerves have little or no spontaneous activity, the sensory nerves very pronounced spontaneous activity.

The motor fibres show a tendency to grouped action leading to composite spikes of constant height, single or repetitive. This is not seen in the same degree in sensory nerves. Grouped action has been studied in both nerve and muscle.

7. Some further correlations between stimulus form and response are:

A rise of the stimulus above the threshold leads to a repetitive response during the rising phase. Its frequency depends upon stimulus gradient and stimulus strength.

By carefully adjusting stimulus strength it is possible to obtain a single response even with very slowly rising stimuli, but with such stimuli there is a tendency of the nerve to respond repetitively.

Repetitiousness during the rising phase of the stimulus has been found to be a different phenomenon from the prolonged repetitiousness during the horizontal level of stimulation.

8. The spike potentials elicited by very rapidly rising stimuli have been subjected to a special analysis with a single sweep arrangement. It has been possible to demonstrate a shortening of the latent period of the impulse with suprathreshold stimuli as is known from experiments on stimulation with instantaneously rising currents.

9. The repetitive response at the horizontal level of the accommodation curve and to constant currents has been analyzed. This response, the prolonged repetition, has the following properties:

The frequency of the response is not determined by the refractory period of the nerve but by current strength. It increases with current strength.

The duration of the rhythmic discharge also increases with current strength.

The nerve shows adaptation to stimulation with constant current. This leads to a gradually decreasing frequency of the discharge.

It is pointed out that the autorhythmic mechanism of the nerve has properties which agree with those of sense organs, and that a nerve stimulated by slowly rising currents is a good model of how a sense organ, such as the retina, may activate its afferent nerve by a generator potential.

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THE PART PLAYED BY THE PYLORIC REGION IN THE CEPHALIC PHASE OF GASTRIC SECRETION

BY

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LUND

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Contents.

Previous experiments.

Since Pavlov's and his coworker's fundamental work on gastric secretion, this is usually divided into three different phases, the cephalic, the gastric and the intestinal phase. The cephalic phase is considered to be initiated and maintained by impulses in the vagus nerves. The gastric and possibly also the intestinal phase — the nature of which is still very incompletely investigated — is considered to be controlled by a combined neuro-humoral mechanism, which sets in at the contact of the food with the gastric and intestinal mucosa respectively.

The cephalic phase of gastric secretion.

That the vagus nerves carry secretory impulses to the HCl- and peptic glands of the gastric mucosa was first shown by Pavlov and Schumov-Simanovski (1895). On sham feeding of gastric fistula dogs abundant gastric secretion was obtained, which completely ceased after bilateral vagotomy. Electrical stimulation of the vagus nerves in unanaesthetized gastric fistula dogs caused secretion of gastric juice containing HCl and pepsin. In experiments on spinal dogs Uschakov (1896) confirmed these results and also claimed to have demonstrated that the mucous glands were vagally innervated.

Since these fundamental researches, the problems of the nervous phase of gastric secretion have been tackled only by a few workers. Not until recent years has the study of gastric secretion excited by electrical stimulation of the vagus nerves been taken up again, in the attempt to analyze in detail the vagal secretory mechanism. Vineberg (1931) stimulated the

vagus nerves of dogs with electrical stimuli of varying intensity. The stronger the stimuli used, the less was the mucus and the higher the acidity and peptiv activity in the gastric juice. This was taken to indicate that the vagus nerves contained different fibres innervating different cytological elements of the gastric mucosa. Bowie and Vineberg (1935) showed, also in dogs, that prolonged vagal stimulation exhausted the store of pepsinogen granules in the peptic glands almost completely, whereas histamine had no effect on the pepsinogen picture.

In the last few years the question how the secretory impulses of the vagus nerves are mediated has come to the fore. The occurrence of a humoral transmitter has been discussed. In gastric juice obtained after histamine and pilocarpine Bloch and Necheles (1938) demonstrated the occurrence of a substance that was thought to be identical with acetylcholine. According to them, this argued for acetylcholine being the final stimulating factor. The view has also been put forward that histamine plays a part in the cephalic secretory mechanism. On vagal stimulation in dogs and cats MacIntosh (1938) and Emmelin et *al.* in some of their experiments observed an increased histamine content in the venous blood or plasma from the stomach. These authors also demonstrated the occurrence of free histamine in gastric juice obtained by vagus stimulation. Babkin (1938/39) is definitely convinced that histamine plays a part in the cephalic phase, though he makes no suggestion as to the mode of its action.

The gastric phase of gastric secretion.

Inspired by Bayliss and Starling's discovery of secretin in 1902, Edkins (1906) postulated the occurrence of a hormone »gastrin» which he thought would play a similar part in stimulating the gastric phase of secretion as does secretin in the secretion of pancreatic juice. In extracts from the mucosa of the pyloric and cardiac regions from dogs and cats, he was able to demonstrate a factor that excited gastric secretion when administered intravenously to anaesthetized cats. The most active extracts were obtained by boiling the mucosa in water or

in 0.4 % HCl. No such factor could be extracted from the fundic mucosa.[1]

Since then a large number of experiments have been carried out in attempts to demonstrate a secretory excitant in the blood during gastric digestion. The results obtained are not uniform. In experiments on cats gastric secretion did not occur on transfusion of blood from fed to fasting animals (LIM 1923). IVY, LIM and MACCARTHY (1925 a) also obtained negative or doubtful

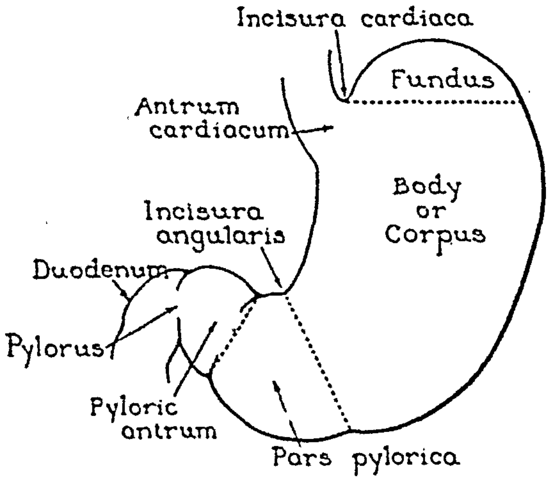

Fig. 1. The parts of the stomach. (After LEWIS.)

results with blood transfusion or cross-circulation in Pavlov fistula dogs. Contrary to this, by transferring defibrinated blood from digesting animals, RASENKOV (1925) observed secretion in fasting Heidenhain fistula dogs. On transfusion of blood from fasting animals however no gastric juice was obtained. The definite proof of the occurrence of a humoral factor was provided by IVY and FARRELL (1925). These authors autotransplanted gastric mucosa to the mammary region of a dog and observed secretion in this completely denervated transplantate 1 to 3 hours after feeding the animal. In vivi-dialysis

[1] In older works and still in many clinical investigations the term fundus is used to indicate the HCl and pepsin producing parts of the stomach. The more recent terminology as used in current papers is somewhat confusing. In the experimental part of this paper the nomenclature given in fig. 1 is employed.

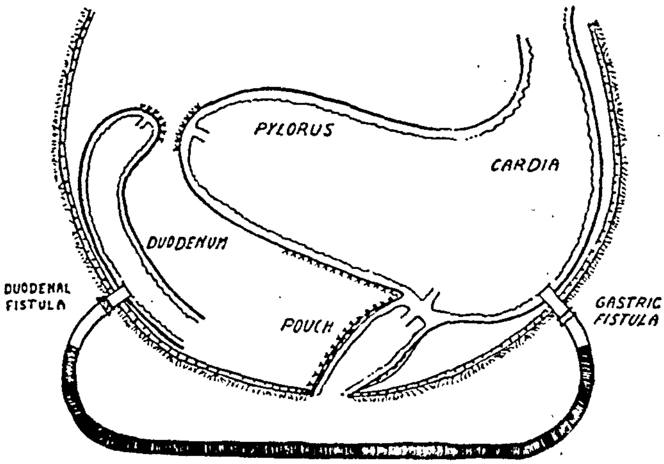

Fig. 2. Diagram of the gastro-intestinal tract of a dog with a gastric *fistula*, *Pavlov pouch, duodenal fistula and the stomach separated from the duodenum at the pyloric sphincter* (from BABKIN 1934).

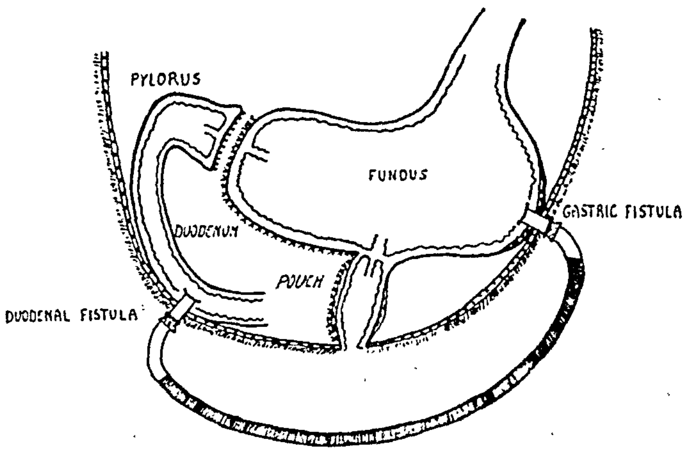

Fig. 3. Diagram of the gastro-intestinal tract of a dog with a gastric fistula, Pavlov pouch, duodenal fistula and the stomach divided into two parts at the bundary between the fundus and the pyloric part (from BABKIN 1934).

of the blood of a fed dog, LIM and NECHELES (1926) observed the appearance of a secretory factor in the dialysate.

The occurrence of a gastric secretory excitant in the blood during the gastric phase thus seems established, although there is uncertainty as to the origin, nature and action of this factor. Many facts suggest that the humoral mechanism is initiated by

some process in the mucosa of the pyloric region. In Pavlov's laboratory it was shown long ago that in dogs with a gastric fistula, Pavlov pouch, duodenal fistula and the whole stomach separated from the duodenum (v. fig. 2), the administration of various food substances through the gastric fistula excited secretion in both the Pavlov pouch and in the whole stomach. In dogs with a gastric fistula and Pavlov pouch but with the fundus disconnected from the pyloric region (v. fig. 3). GROSS (1906) found that no secretion occurred if food was introduced

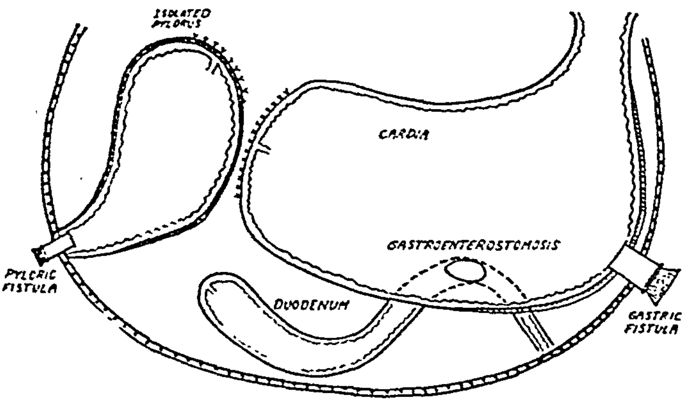

Fig. 4. Diagram of the gastro-intestinal tract of a dog with a gastric fistula, gastro-enterostomy and an isolated pyloric pouch as used by Savitsch and Zeliony (from BABKIN 1934).

through the gastric fistula. When introduced in this way alcohol and histamine were the only agencies which excited secretion. If however the various food substances were administered through the duodenal fistula, gastric juice was secreted in the fundus.

GROSS concluded from these results that the gastric phase was stimulated via the pyloric region. EDKINS and TWEEDY (1909) and SAVITSCH and ZELIONY (1913) also observed gastric secretion when secretagogues [1] were introduced into an isolated pyloric pouch (v. fig. 4). No secretion was obtained if these

[1] According to Ivy, the term secretagogue will be used to designate those substances present in food or produced by the digestion or decomposition of food that excite the secretion of digestive juice by acting locally or by being absorbed in the blood or lymph or by causing a hormone to be formed.

substances were brought into contact only with the fundic mucosa. LIM, IVY and MACCARTHY (1925) were able to demonstrate secretion in the fundic mucosa as a result of mechanical as well as chemical stimulation of an isolated pyloric pouch. These authors were also able to induce a slight secretion in the fundic mucosa by direct mechanical stimulation of the fundus but not by chemical stimulation. Contrary to this, IVY and WHITLOW (1922) were unable to demonstrate any secretion in the Pavlov pouch after application of various food substances in an isolated pyloric pouch. In similar experiments PRIESTLY and MANN (1932) report doubtful or negative results. STEINBERG et *al.* (1927) also obtained somewhat conflicting results. They observed in dogs a scanty HCl-secretion on direct application of meat extract to the fundic mucosa.

In a large number of experiments on animals and in clinical investigations the gastric secretion has been studied after various operative procedures on the stomach. The results obtained are very hard to analyze. Some authors give no informations about the test meal applied. In some experiments only the acidity, in others only the secretory volume was studied. In some reports the secretion is examined during 1 to 2 hours after the test meal, in others during longer periods, whereas in a third group of reports no time relations whatever are stated. From such reports no definite information about the cephalic, gastric and intestinal phases of secretion in the stomach operated on can be obtained.

After resection of the pyloric region in intact dogs and in dogs with whole stomach pouches, WILHELMJ et *al.* (1936) found a considerable decrease of secretory volume after a test meal. It dropped to $^1/_5$—$^1/_6$ of previous values, whereas the acidity remained unchanged. After total resection of the pyloric region in dogs MACCANN (1929) was unable to demonstrate any free HCl after a test meal. If however parts of the pyloric mucosa were spared there was no change in the acidity.

Resection of the pyloric region in Pavlov fistula dogs was carried out by SMIDT (1923), PORTIS and PORTIS (1936), STEINBERG et *al.* (1927) and SHAPIRO and BERG (1934) and the secretion following test meals studied. SMIDT found a considerable decrease

of secretion from the Pavlov pouch, chiefly during the gastric phase but also during the psychic phase. PORTIS and PORTIS report that after the resection no free HCl could be observed in the residual stomach, whereas the combined acidity was high. The Pavlov pouch still showed secretion. STEINBERG et *al.* observed a decreased rate of secretion after the resection, whereas SHAPIRO and BERG report a decreased acidity which returned to normal after 3—6 weeks. In Heidenhain fistula dogs GRINDLEY (1941) found that resection of the pyloric region had no effect on the total secretion of the pouch measured during the course of 24 hours after the test meal. WANGENSTEEN et *al.* (1940) report unchanged secretory conditions in Pavlov fistula dogs after resection of the pyloric region. In these experiments gastric secretion was excited by alcohol or histamine.

An interesting observation was made by KLEIN (1935). The secretion after a test meal in the fundic pouch of a dog, autotransplanted subcutaneously, disappeared after resection of the pyloric region. LEWIS (1938) excised the mucosa of the pyloric region in dogs, leaving the muscular wall between the stomach and the duodenum intact. In four cases a marked hypoacidity still persisted after 8—10 months. In two cases where on microscopic examination slight fragments of the mucosa from the pyloric region were observed, only a slight decrease in the acidity occurred.

In a clinical investigation on patients with their pyloric regions resected SCHUR and PLASCHKES (1915) found after a test meal free acidity in only one of six cases. In a group of 44 patients with complete resection of the pyloric region LORENZ and SCHUR (1922) observed only one patient with free HCl. In patients with only partial resection free HCl appeared in 11 cases out of 12. In a similar group of patients, after a Rehfuss test meal KLEIN (1927) observed a large number of cases with anacidity during the first 1 to 2 hours. Secretion of HCl often occurred at a later stage when the food had already left the stomach. Of sixty patients with their stomach operated on by various procedures, WANGENSTEEN et *al.* (1940) found in patients with the pyloric region resected no decreased secretory response to alcohol and histamine.

The data so far derived from experimental and clinical observations indicate definitely that gastric secretion may persist after resection of the pyloric region. The observations suggest however that this region plays an important part in controlling the gastric phase. Whether the humoral factor liberated from the pyloric region is to be regarded as a hormone or as a secretagogue is still a matter of dispute.

MAYDELL (1913) like LIM (1923) confirmed EDKINS statement that HCl-extracts from the pyloric and the cardiac mucosa contained a secretory excitant, whereas extracts from the fundic mucosa were inactive. POPIELSKI (1909), like EMSMANN (1912), EHRMANN (1912) and others showed that HCl-extracts from various mucous regions in the alimentary canal and many tissue extracts induced gastric secretion on intravenous and subcutaneous administration. By various methods of extraction KEETON and KOCH (1915), LUCKHARDT et *al.* (1920) demonstrated »gastrin bodies» in extracts from many tissues and organs.

DALE and LAIDLAW (1910) assumed that the active principle in the extracts of POPIELSKI et *al.* was histamine. In 1920 POPIELSKI demonstrated the gastric secretory effect of histamine. The problem whether histamin was »the gastric hormone» became very absorbing. SACHS, IVY, VANDOLAH and BURGESS (1932) obtained crystalline histamine from extracts of the pig's pyloric mucosa. These investigators claimed that their experiments offer strong evidence in favour of the view that in acid extracts of the pyloric mucosa histamine is the sole secretory excitant which is active when the extract is introduced subcutaneously. The authors continue, »It has not been proved that histamine is a gastric hormone, neither has it been proved that there is a gastric hormone».

It is a remarkable fact that according to GAVIN, MCHENRY and WILSON (1933) the fundic region contains most of the histamine of the gastric mucosa, a fact that has been confirmed in cats and dogs by EMMELIN et *al.* and others.

KOCH, LUCKHARDT and KEETON (1920) argue that their »gastrin bodies» are not identical with histamine, but seem to be a closely related imidazol derivate. As already pointed out by EDKINS his preparations of »gastrin» showed vasodilator properties; the

secretory inactive fundic extracts exerted even stronger vasodilator effects. Working in Babkin's laboratory quite recently Komarov (1938) claims to have obtained from the pyloric mucosa of dogs a histamine-free extract which on intravenous injection excited gastric secretion in cats. Active extracts were also derived from the duodenal mucosa, especially from the proximal parts, whereas extracts from the fundic or the jejunal mucosa were quite inactive. The active extracts showed no vasodilator properties. In Ivy's laboratory these results could not be confirmed (Ivy 1941).

The gastric phase can be excited without the cooperation of extragastric nerves. In exciting the secretion local nervous structures however seem to play an important part. Zeliony and Savitsch (1911/12) and Savitsch (1922) established that after cocainization of the mucosa of an isolated pyloric pouch, secretion could no longer be obtained in the fundic region on introduction of various food substances into the pyloric pouch. They hypothesized that on paralysing afferent nerve endings a stimulating reflex was inhibited. These investigators like Lim, Ivy and MacCarthy (1925) later, showed further that the gastric phase was also inhibited by injection of atropine. From these experiments Ivy (1930) concludes that postganglionic vagal fibres are necessary for excitation of the gastric phase. According to this author these facts suggest »two mechanisms concerned; a nervous (vascular or secretory) and a hormone, and that the formation of the hormone is dependent to a great extent on the integrity of a nervous mechanism susceptible to atropine. Orbeli's (1906) results favour the view that an intact innervation to the glands is essential for their secretory activity. He found that after denervation — i. e. after transformation into a Heidenhain pouch — a Pavlov pouch showed a successively decreasing secretory activity. According to Orbeli this is due to a diminished excitability of the glands following denervation. No atrophy of the glands could be observed on microscopic examination one year and nine months after the denervation.

All these results suggest that probably a hormone as well as a local nervous mechanism are concerned in stimulating the gastric phase. According to Babkin (1938/39) the picture is

still more complicated. Supported by experimental observations, he points out that digestive products may be absorbed into the blood stream and stimulate the gastric glands directly or via the secretory vagal centres in the brain which according to him are sensitive to such substances.

The intestinal phase of gastric secretion.

The nature of the intestinal phase of gastric secretion is still practically unknown. This phase of secretion was discovered by PAVLOV and his *co-workers* (PAVLOV 1898). IVY, LIM and MACCARTHY (1925 b) worked on whole stomach pouch dogs, where direct connection was established between the oesophagus and the duodenum and the whole stomach formed an isolated pouch. 1 to 3 hours after feeding such animals secretion started in the isolated whole stomach. The oral administration of unabsorbable substances such as saponins also led to secretion which could be stopped by small doses of atropine.

The significance of the intestinal phase is stressed by CRIDER and THOMAS (1932), ENDERLEIN and ZUCKSCHWERDT (1931), WILHELMJ et *al.* (1936) etc. If in Pavlov fistula dogs the intestinal phase was excluded by draining the gastric contents past the duodenum the secretion in the Pavlov pouch according to CRIDER and THOMAS decreased to about $^1/_3$.

The present experimental evidence favours the view that the nervous (cephalic) phase and the two chemical (the gastric and the intestinal) phases are excited by different mechanisms, the former by secretory impulses via the vagus nerves, the two latter by a combined neuro-humoral mechanism. Some clinical and experimental observations however point to a more intimate connection between the three phases.

Some clinicans have been struck by the fact that after resection of the pyloric region the nervous phase of the gastric secretion disappeared (GOETZE (1927), SCHUR and PLASCKES (1915) and others). No explanation of this could be offered.

As far as we can see the rôle of the pyloric region in the cephalic phase of gastric secretion has not been examined by previous workers except for a paper by STRAATEN (1923). He showed in dogs that the gastric secretion obtained on sham feeding practically disappeared after resection of the pyloric region. After complete resection the lack of secretion was permanent, whereas following incomplete resection the nervous secretion gradually returned during the course of 6 to 8 months. From these results STRAATEN suggests the possibility of a common mechanism for the gastric and the cephalic phases.

Experimental technique.

All experiments were performed on cats and dogs from which food had been withheld during the preceding 24 hours. The anaesthetic used was a mixture of chloralose and urethane. During a short ether anaesthesia 0.05 gm. chloralose and 0.5 gm. urethane per kg. body weight were slowly injected intravenously. Before anaesthesia the dogs received 10 mg. morphine per kg. body weight subcutaneously.

Operative procedure.

The vagus nerves were exposed in the neck from as proximal as possible downwards to the thorax aperture. In cats the vagi were separated from the sympathetic trunks. Both vagi were cut, whereas the sympathetic trunks were left intact. In dogs the vagal-sympathetic trunks were freed and bilaterally cut. The trachea was cannulated. Artificial respiration was resorted to only in some experiments, where the administration of cocaine caused respiratory paralysis. In some experiments the oesophagus was ligated, in the others the animal was placed with its head low to prevent saliva from running down into the stomach.

Technique employed in collecting gastric juice.

After opening the abdomen a short longitudinal incision was made in the ventral wall of the stomach just proximal to the pyloric region. A glass or metal cannula to which a rubber tube was attached was introduced through this incision and tightening achieved by sutures. The tip of the cannula was

pushed through an incision in the left abdomen and the abdominal wall closed. To avoid regurgitation of intestinal contents the duodenum was ligated just proximal to Vater's ampulla. The animal was placed on its left side, the front half of the body somewhat higher to facilitate the outflow of the gastric juice.

Technique employed in nerve stimulation.

The vagus nerves were stimulated by platinum electrodes of the Palmer type. At the beginning the electrodes were always applied at the central end of the severed vagi and during the course of the experiment they were gradually moved peripherally to eliminate the effect of local injuries under the electrodes. During the experiment the nerves were kept moist by cotton pellets moistened with Tyrode solution.

The electrical stimulus was derived from a generator yielding alternating current of about 40 periods per second. The intensity varied between some tenths of a milliampere and 3 milliamperes. Rhythmical alternating stimulation of the vagus nerves was arranged for by attaching into the circuit a metronome with a frequency of about one stroke per second. In cats the vagus nerves were stimulated continuously by this technique during several hours. In dogs, where the secretion decreased already after some minutes of stimulation, the nerves were stimulated for periods of 2 minutes and allowed to rest 1 minute in between.

The rate of secretion was generally measured in 15-minute periods. The gastric juice was tested for free and combined acidity.

Determination of acidity.

Free and total HCl were determined by titration electrometrically, a potentiometer according to Prahn being used. The indicator electrode was a fine silver rod in a thin-walled glass ballon filled with N/10 HCl saturated with AgCl. The other half element consisted of saturated KCl (Hg_2Cl_2) Hg with a connecting link of saturated KCl.

The glass electrode was mounted so that it could be raised and lowered during titration. During this the content was mixed in the titration vessel by bubbles of air from a capillary tube fused in the bottom of the vessel. The air was first led through 10 N NaOH and $CaCl_2$. The potentiometer was controlled several times daily against buffer solutions with known pH.

Titration was carried out on 2 cc. of gastric juice with N NaOH from an 1-cc-burette graduated in 0.01 ccs. The capillary point of the burette dipped into the contents of the titration vessel during titration.

pH 3.5 was taken as the limit for free HCl and pH 7.4 for the total HCl. The HCl values were given in milli equivalents per litre (m. e./lit.). In 20 titrations of the same gastric juice the standard deviation for the determinations of free HCl amounted to 3.3 m. e./lit. and for those of total HCl to 4.8 m. e./lit.

Own experiments.

On sham feeding of dogs gastric juice of high acidity and high peptic activity is secreted within 5 minutes (PAVLOV and SCHUMOV-SIMANOVSKI). Contrary to this the secretory response to electrical stimulation of the vagus nerves is often poor and delayed, the juice being mucous and of low acidity (PAVLOV and SCHUMOV-SIMANOVSKI, USCHAKOV, VINEBERG). Frequently the secretion does not start until 30 to 60 minutes after the beginning of stimulation (USCHAKOV, BOWIE and VINEBERG). This long latency is considered by USCHAKOV and by BABKIN (1928) to indicate the existence of inhibitory fibres in the vagus nerves. Not until these fibres are eliminated due to their greater liability to fatigue does stimulation of the vagi cause gastric secretion.

At the beginning of our own experiments difficulties were encountered in obtaining satisfactory gastric secretion on electrical stimulation of the vagus nerves in the cat. Attempts were made to investigate if this was actually due to stimulation of inhibitory fibres as suggested by BABKIN. The stimulating electrodes were placed in various sections of the vagi from the nodose ganglion downwards to just above the diaphragm. No secretion or only a scanty flow was obtained during several hours stimulation with a current intensity of several milliamperes which caused a pronounced vagal effect on the heart. In a series of experiments the vagus of one side was cut and time allowed for degeneration — it is generally assumed that inhibitory fibres degenerate prior to excitatory fibres. Four to seven days after section of the nerve the peripheral end of the vagus was stimulated. The secretory response however remained poor.

Attempts were then made to stimulate secretory fibres selectively by varying the strength, steepness and frequency of the stimulus. The amounts of gastric juice obtained however were poor.

In all these preliminary experiments a ligature was tied around the pylorus to prevent the regurgitation of intestinal contents and the escape of gastric juice into the duodenum. If the pylorus was left untouched and the ligature tied around the duodenum just proximal to Vater's ampulla, large quantities of gastric juice were obtained on vagus stimulation. This observation was the impetus to the experiments now to be described.

The normal process of secretion.

In almost all experiments the stomach of fasting anaesthetized animals contained gastric juice in varying quantities. In cats the content was seldom less than 1 to 2 cc. Usually it amounted to about 5 cc. and in exceptional cases to over 10 cc. The juice was watery and the acidity was generally low. The stomach of the dogs also contained gastric juice in quantities usually amounting to several tens of cc.

On vagus stimulation with a latency of only a few minutes gastric juice emerged from the cannula. During the stimulation the rate of secretion increased successively and usually reached its maximal value 15 to 30 minutes after the beginning of the stimulation. From then onwards abundant secretion could be maintained during several hours' stimulation. The secretion showed great individual variations. Fig. and table I demonstrate some characteristic features of the course of secretion. In this experiment the secretion differs from the usual standard type in that during the first 15 minutes it was unusually abundant. It is seen from this figure that abundant secretion can be maintained during nearly five hours' stimulation and that the speed of secretion decreases somewhat during the later stage of the experiment.

The first drops of gastric juice were often mucous and the acidity in the first portion of gastric juice was low. Not until

about 30 minutes after the stimulation was started did the acidity rise to a level that was afterwards maintained relatively unchanged during the whole process of secretion. During the course of several hours' stimulation the acidity showed a tendency to decrease.

Secretion after ligation of the pylorus and its blood vessels.

a. Secretion after ligation around the pylorus.

The blood supply to the pyloric region is derived from arteries running along the curvatures. These blood vessels

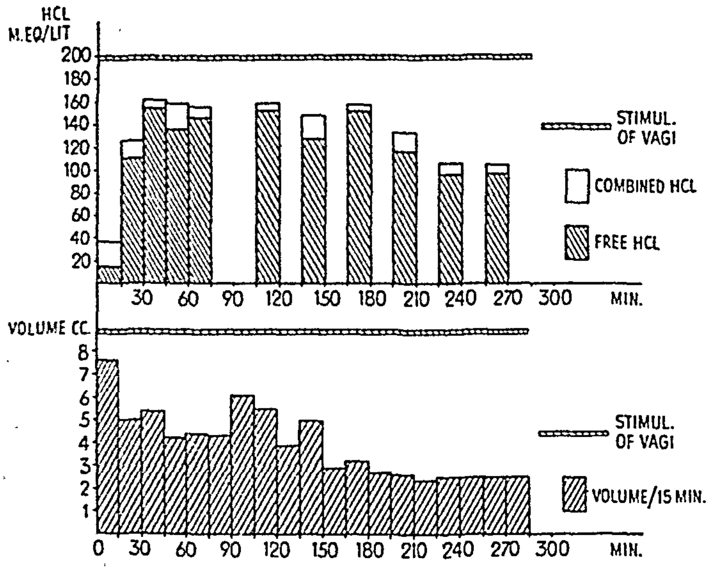

Fig. I. Cat. Gastric secretion during stimulation of vagi (for details see table I).

usually join the curvatures at or close to the pylorus. The observed inhibitory effect of the pyloric ligature may be due to obstruction of the blood supply to the pyloric region.

In a series of experiments the ligature was tied around the pylorus so as to include the arteries supplying the pyloric region. Fig. and table II show an experiment of this type, where the ligation was first tied closely proximal to Vater's

ampulla. During the course of two hours, vagal stimulation resulted in secretion of about 30 cc. The ligature was now removed and the pylorus with its arteries ligated. After this procedure stimulation during 2 hours did not produce even 1 cc.

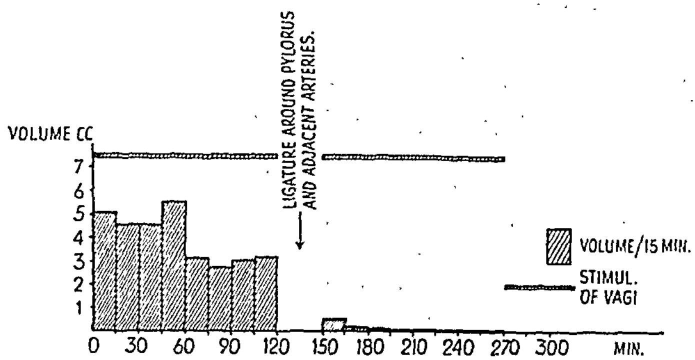

Fig. II. Cat. Gastric secretion during stimulation of vagi before and after ligature around pylorus and adjacent arteries (for details see table II).

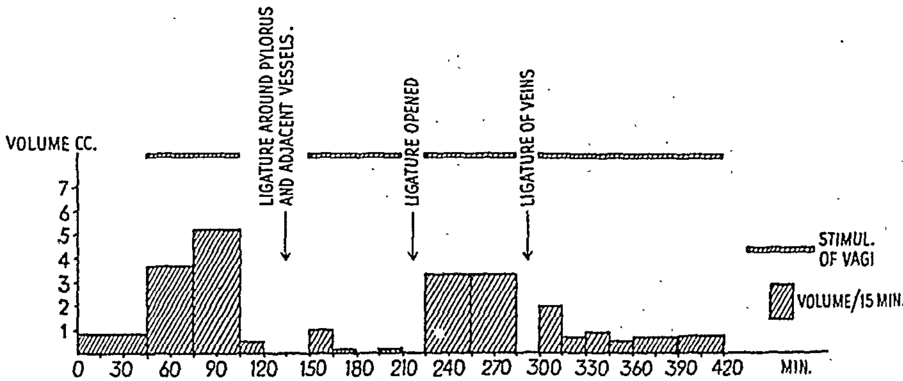

Fig. III. Cat. Gastric secretion during stimulation of vagi before and after ligature around pylorus and adjacent arteries. At a later stage the ligature was removed and following this, only the adjacent veins were ligated (for details see table III).

juice. In 4 experiments of this type, where vagal stimulation excited abundant secretion of watery acid juice, the secretion stopped entirely or declined to an insignificant amount after ligation of the pylorus and its arteries. The scantily secreted juice was highly mucous.

In fig. and table III one more experiment of this type is shown. During about 1 hour's stimulation of the vagi circa

18 cc. watery juice were secreted. After tying the ligature about the pylorus not much more than 1 cc. of mucous juice was secreted during a further hour's stimulation. On removing the ligature the rate of secretion rose again. A second experiment on another cat gave similar results.

In all experiments but one — at present more than 70 — where the ligature was tied closely proximal to Vater's ampulla, abundant gastric secretion was obtained on stimulating the vagi.

b. Secretion after ligation of the arteries to the pyloric region.

The two main arteries supplying the pyloric region are branches of the hepatic artery. In two cats where the two

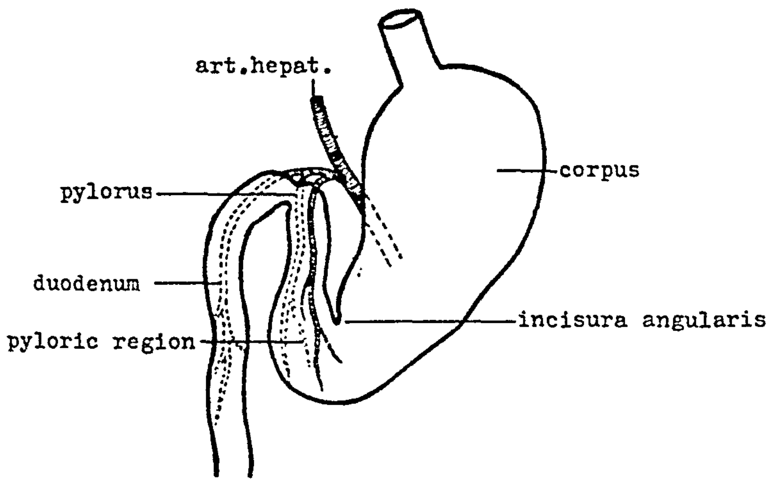

Fig. 5. Schematic representation of the arterial blood supply to the pyloric region in the cat.

arteries left the hepatic artery with a common trunk this was ligated (v. fig. 5). Before ligation abundant secretion of watery acid juice was obtained. In the experiment shown in fig. and table IV during the first hour of stimulation 37.5 cc. were secreted. After ligature of the arterial trunk the secretion decreased abruptly and during the following hour only circa 8 cc. juice were secreted. Coincidently with the decrease the juice changed character and became highly mucous and poor

in HCl. During the later course of stimulation the juice again became less mucous and the acidity rose.

c. Secretion after obstruction of the venous flow from the pyloric region.

Similar results were obtained on obstruction of the venous flow from the pyloric region (v. fig. and table III). After one hour's stimulation during which 13.6 cc. watery juice were

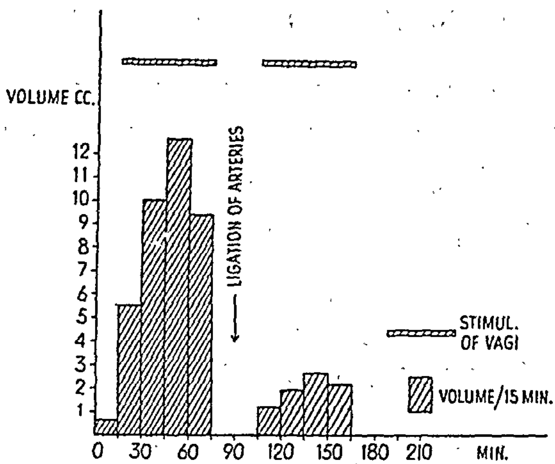

Fig. IV. Cat. Gastric secretion during stimulation of vagi before and after ligation of arteries to pyloric region (for details see table IV).

secreted, the veins of the pyloric region were ligated close to the pylorus. The secretion then decreased considerably, and during the following hour only 4.1 cc. juice was obtained. Having been watery and strongly acid, the juice became mucous and poor in HCl after tying the veins. Another experiment of this type is shown in fig. and table V. On stimulation of the vagi during 2 hours circa 19 cc. gastric juice were secreted. After obstruction of the veins with an arterial clip the secretion decreased and amounted to only 3.1 cc. during 1 hour's stimulation. On opening the veins the secretion rose to double. The gastric juice secreted prior to the venous obstruction was of relatively high acidity. After venous obstruction the acidity declined and again rose in opening the

veins. In a third experiment of this type secretion stopped altogether after ligation of the veins.

The mucosa of he pyloric region in cats and dogs contains only a relatively small number of HCl- and peptic glands. According to LIM (1922) in a zone 35 mm. broad reckoning from the pylorus, there exist in cats only »pyloric» cells and outside this boundary a gradual transition to a parenchyma containing HCl- and peptic glands is seen. Obviously the obstruction of the

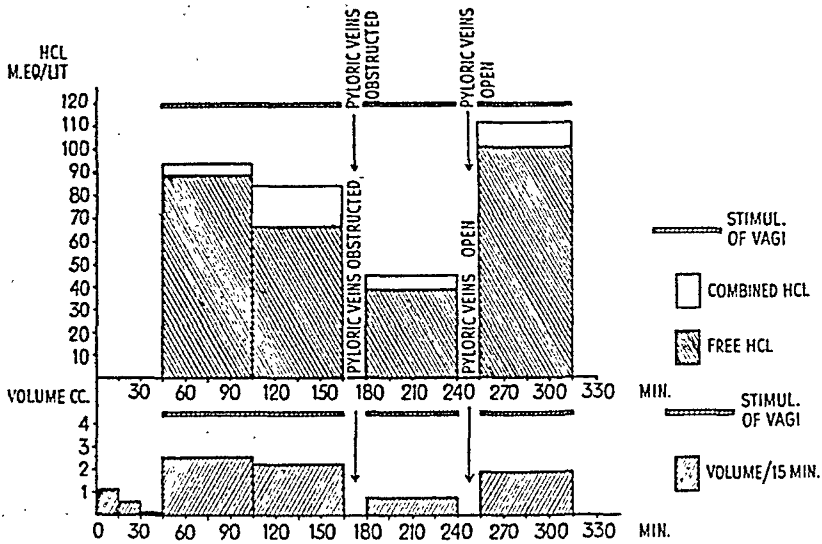

Fig. V. Cat. Gastric secretion during stimulation of vagi before and after ligation of veins from pyloric region (for details see table V).

pyloric vessels is not likely to interfere appreciably with the blood supply of the HCl- and peptic cells.

From these experiments we hypothesized that in the pyloric region some stimulating agent is liberated into the blood stream during stimulation of the vagi.

Secretion after cocainization of the pyloric mucosa.

ZELIONY and SAVITSCH were able to excite secretion in the fundic mucosa by introducing various substances into an isolated pyloric pouch. After cocainization of the pyloric region there was no secretion in the fundus.

In a series of experiments we cocainized the pyloric mucosa in cats and stimulated the vagi. After a period of vagal stimulation the abdomen was opened, the gastric cannula was removed, and the pyloric mucosa was rubbed with a 2 % cocain solution during about 5 minutes. The mucosa was then wiped dry, the gastric cannula again inserted, and the vagal stimulation resumed. Fig. and table VI show an experiment of this type. The vagi were stimulated during 60 minutes and about 16 cc. of acid watery juice obtained. After cocainization of the pyloric

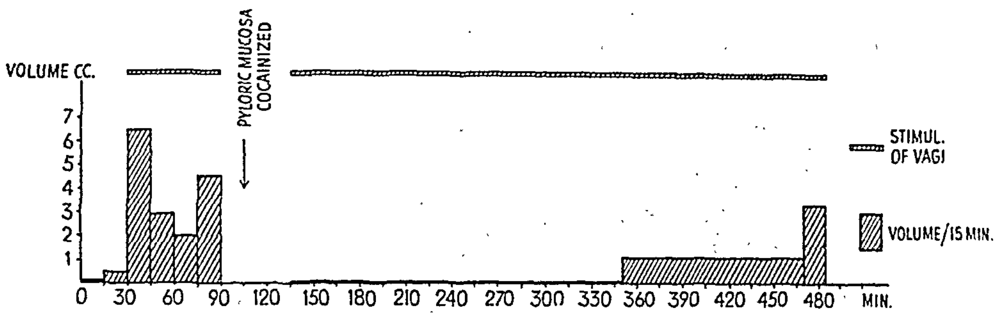

Fig. VI. Cat. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa (for details see table VI).

mucosa the vagi were again stimulated after a resting period of 45 minutes. Although the strength of the stimulating current was increased from 0.5 m. a. to 2 m. a. only a few drops of mucus were secreted during the next 3.5 hours. Later on the secretion again increased; not until 6 hours after the cocainization did the rate of secretion return to normal.

Experiments of this type were performed in 20 cats. The results were quite uniform. Usually 2 % or 3 % cocaine solution was used. In some cases, where an initial treatment with 2—3 % solution was ineffective, the secretion stopped after an additional treatment with 5 % cocaine. Further data from these experiments are presented in figs and tables XIII, XIV, XVI, XVIII, XIX and XXI.

In 2 experiments cocainization of the pyloric region did not interfere with the secretory effect of vagal stimulation.

Cocaine does not obstruct the secretory mechanism by paralysing the HCl- and peptic cells. This is evident from

experiments yet to be described, where histamine and other active agents caused secretion in cats after cocainization of the pyloric mucosa. A considerable absorption of cocaine actually occurs however from the pyloric region; in some

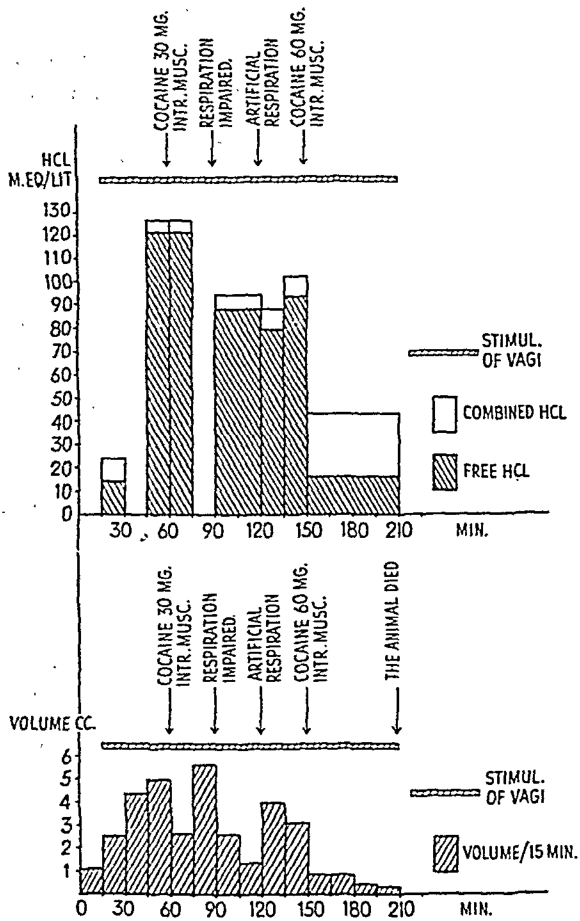

Fig. VII. Cat. Gastric secretion during stimulation of vagi before and after injection of cocaine intramuscularly (for details see table VII).

experiments respiratory paralysis resulted and the animal died, if artificial respiration was not resorted to. It was difficult to evaluate how much of the cocaine entered the blood stream after cocainization of the pyloric mucosa. In order to get some information about the effect of cocaine on gastric secretion the vagi were stimulated after cocaine had been injected

intramuscularly. One of these experiments is shown in fig. and table VII, where after 45 minutes' stimulation 30 mg. cocaine was given intramuscularly. The cat became asphyctic and the gastric secretion decreased. On initiating artificial respiration the secretion increased to almost the previous rate. After a second dose of 60 mg. cocaine intramuscularly, the secretion again decreased and symptoms of serious intoxication occurred. Three experiments of this kind showed that injection of cocaine in doses sufficient to cause respiratory paralysis only slightly diminish the secretory response to vagus stimulation.

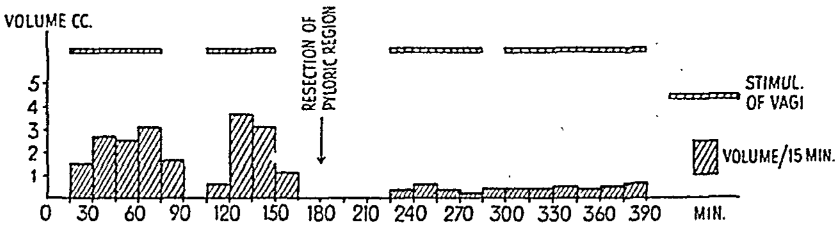

Fig. VIII. Cat. Gastric secretion during stimulation of vagi before and after resection of pyloric region (for details see table VIII).

From these experiments it seems justifiable to conclude that the inhibitory effect of cocaine when applied to the pyloric mucosa is of local origin. These experiments support the idea that on vagus stimulation some active agent is liberated in the pyloric region. Strong evidence supporting this theory is presented in the following experiments.

Secretion after resection of the pyloric region.

In a first group of experiments the pyloric region was resected in cats. The resection included a distal part of the stomach from closely proximal to incisura angularis to just distal to the pylorus. Fig. and table VIII show an experiment of this type. Before the resection vagal stimulation during 60 minutes yielded 9.8 cc. gastric juice, and during a second period of stimulation 8.5 cc. were obtained. After resection of the pyloric region the secretion decreased considerably. Stimulation during 60 minutes gave only 1.7 cc. juice, and during a

further 90 minutes only 2.8 cc. were secreted. Completely uniform results were obtained in all (9) experiments. After resection of the pyloric region the secretion abruptly decreased in all cases to only some tenths of a cc. per 15 minutes. Some of these experiments are represented in figs and tables XV and XXII.

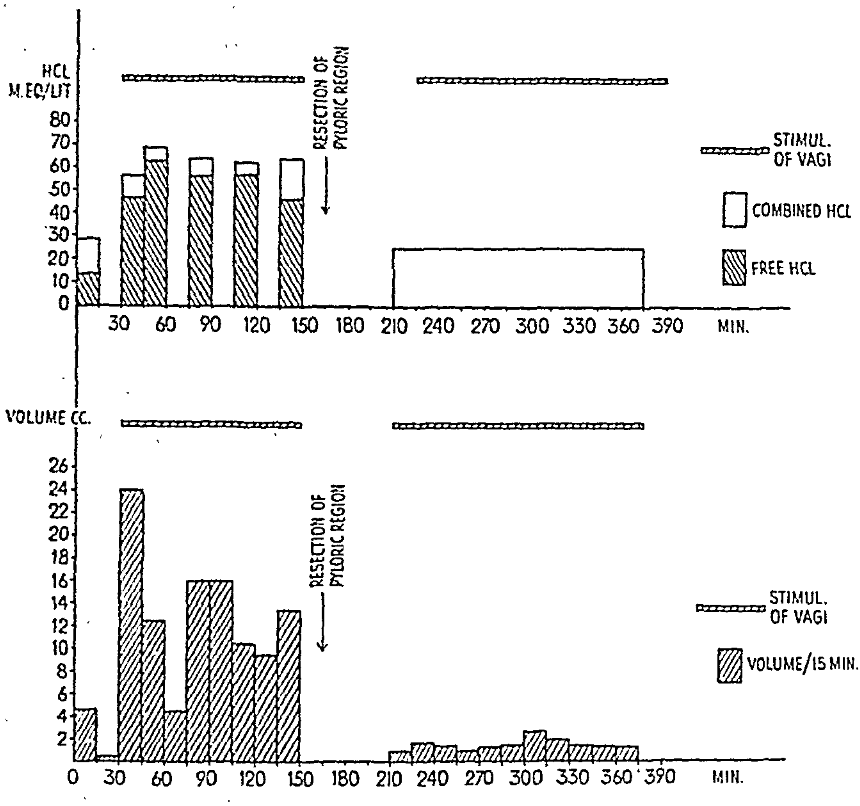

Fig. IX. Dog. Gastric secretion during stimulation of vagi before and after resection of pyloric region (for details see table IX).

The effect of pyloric resection was still more dramatic in dogs. In the experiment seen in fig. and table IX abundant secretion persisted during 2 hour's stimulation. After resection of the pyloric region secretion decreased to a negligible amount, and the gastric juice completely changed character. The juice contained no free HCl and was of a high stringy mucous consistency. Identical results were obtained in 4 other dogs. Two of these experiments are seen in figs and tables XVII and XX.

The depressing effect of pyloric resection can not be due simply to a reduction of the HCl- and pepsin secreting area, since this is only slightly encroached upon by the operation. At the utmost a quarter of the total gastric mucosa is removed by the resection. Neither can the depressing effect be due to operative traumata. This is clear from the experiment referred to in fig. and table IX. This animal was an old gastric fistula dog on which immediately prior to the actual experiment the

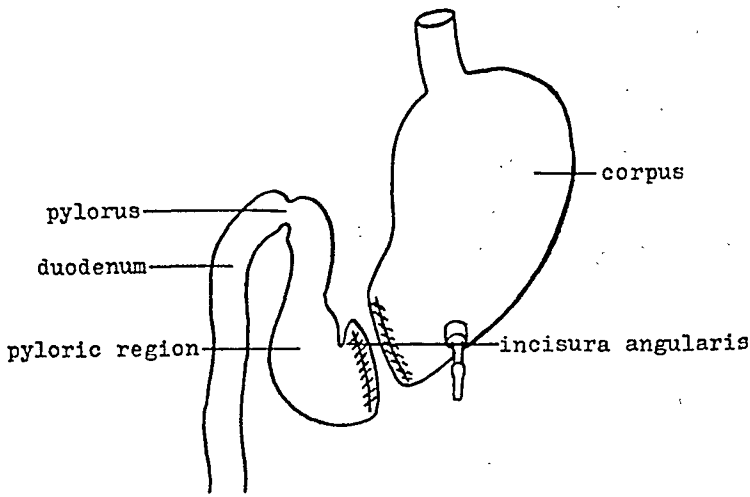

Fig. 6. Technique employed in disconnecting the pyloric region from the stomach.

fistula was extirpated, an operation involving traumata at least as long and severe as the resection at a later stage of the pyloric region. In a second experiment a Pavlov fistula dog was used. In both these dogs very abundant secretion was obtained on vagus stimulation after resection of the minor pouches before resection of the pyloric region.

Secretion after disconnection of the pyloric region from the stomach.

In 5 experiments on cats and 1 on a dog the secretion was investigated after disconnection of the pyloric region from the stomach. This was carried out by a circular incision through the

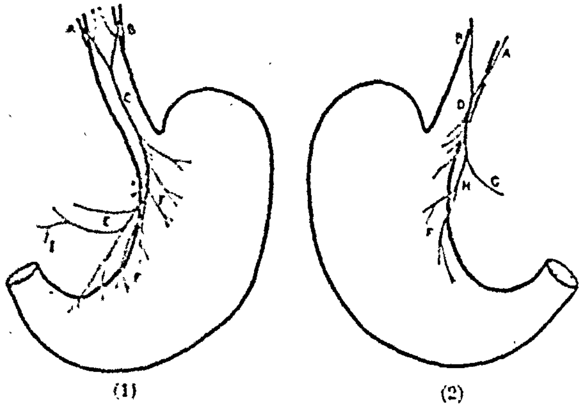

Fig. 7. Drawings illustrating the formation and distribution of the anterior (ventral) and posterior (dorsal) vagal trunks in the cat. The anatomical arrangement in the dog is similar, (1) anterior, (2) posterior. A, right vagus. B., left vagus. C, anterior vagal trunk. D, posterior vagal trunk. E., hepatic branch. F, gastric branches. G, coeliac division. H, gastric division. I, pyloric branch. (From MacCrea 1925.)

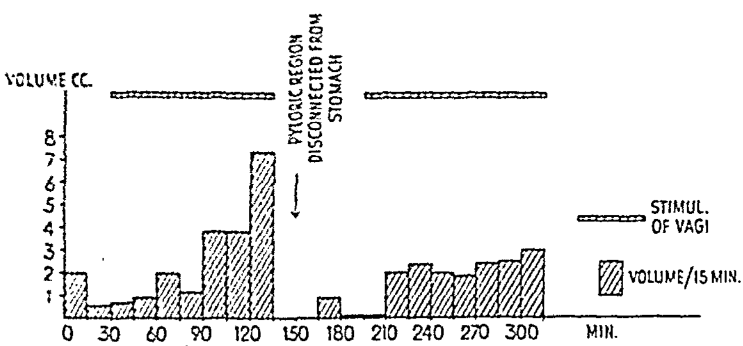

Fig. X. Cat. Gastric secretion during stimulation of vagi before and after disconnecting pyloric region from stomach (for details see table X).

whole stomach wall just proximal to incisura angularis (v. fig. 6). The incision was prolonged through the lesser omentum in order to sever the vagal pyloric fibres which may cross that region (McCrea 1925 v. fig. 7, Stiemens 1934).

In all experiments the secretory response to vagus stimulation declined after disconnection of the pyloric region from the stomach. In one experiment, where the rate of secretion

was rather low, it dropped to zero after the disconnective operation. In all the other experiments a considerable secretion was still obtained during stimulation of the vagi. In three of the experiments the juice acquired a pronounced mucous character after the »denervation». This change however was transient. During later stages of vagus stimulation after

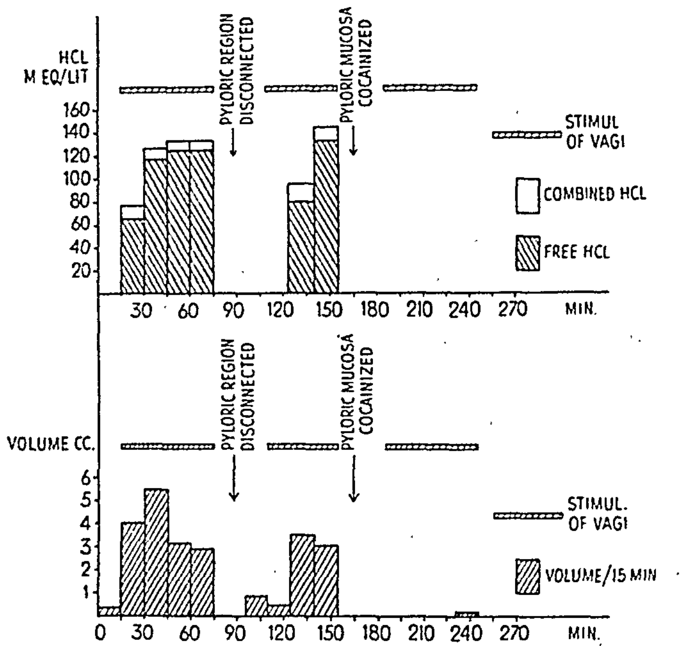

Fig. XI. Cat. Gastric secretion during stimulation of vagi before and after disconnecting pyloric region from stomach. At a later stage the pyloric mucosa was cocainized (for details see table XI).

»denervation» of the pyloric region the juice again became watery. In all experiments the acidity remained high after »denervation». Fig. and table X show an experiment of this type.

In two experiments the mucosa of the disconnected pyloric region was cocainized. The persistent secretion then disappeared completely (v. fig. and table XI). In a third experiment the disconnected pyloric region was resected and following this the secretion disappeared almost completely.

Only one experiment of this type was performed on a dog (fig. and table XII). After disconnection of the pyloric region

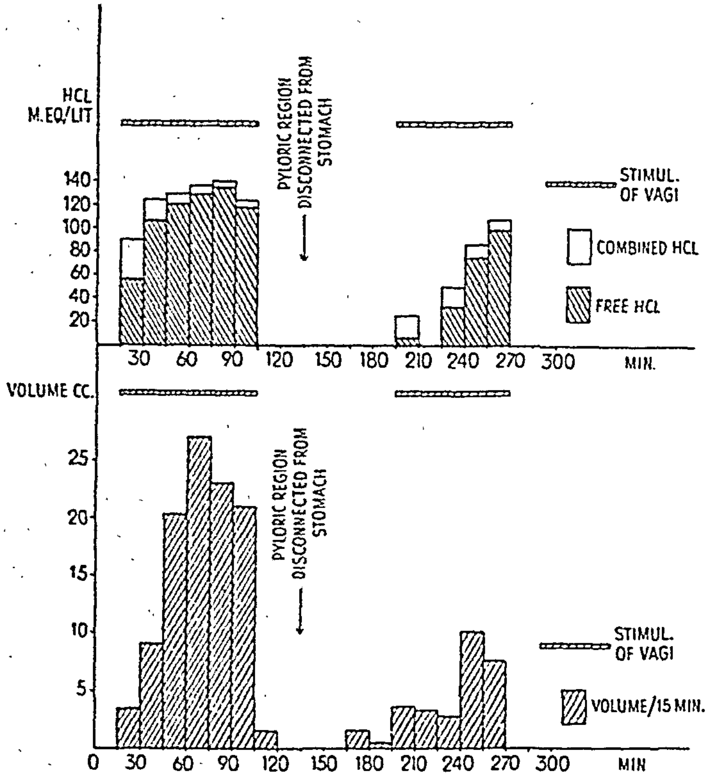

Fig. XII. Dog. Gastric secretion during stimulation of vagi before and after disconnecting pyloric region from stomach (for details see table XII).

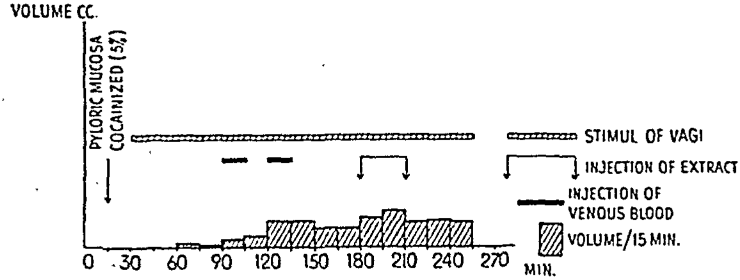

Fig. XIII. Cat. no. 1. Pyloric mucosa was cocainized. Gastric secretion during stimulation of vagi and intravenous injection of venous blood from stomach of cat no. 2. Following this, extract from cat's pyloric mucosa was injected intravenously. Cat. no. 2. Venous blood was drawn from stomach during stimulation of vagi (for details see table XIII).

the secretion became highly mucous in character.. During the later course of the stimulation the juice again became watery and acid as the rate of secretion increased.

The characteristics of the secretion in the animals after disconnecting the pyloric region from the stomach thus differ from those observed after pyloric resection. The experiments further support the idea that the gastric secretion during stimulation of the vagi is secreted by co-operation of some factor emanating from the pyloric region and brought to the gastric glands via the blood stream.

Cross-circulation experiments.

The definite proof of a hormonal mechanism within the cephalic phase of gastric secretion would be provided by the demonstration of a gastric secretory excitant in the blood stream during vagal stimulation. Experiments dealing with this problem will now be described.

In the experiment shown in fig. and table XIII a cat was prepared in the following way. After an adequate gastric secretion had been observed on vagal stimulation during 30 minutes a cannula was inserted into the superior mesenteric vein distally to the inflow of the gastro-splenic vein and with its tip pointing towards the portal vein. A ligature was placed around the portal vein and during vagal stimulation the blood flow was obstructed by pulling the ligature. By this procedure the venous flow from the stomach was diverted to the venous cannula. Clotting was prevented by previous injection of heparine.

In a second cat the pyloric mucosa was cocainized. After the cocainization only a negligible amount of congo-negative gastric juice was obtained during 45 minutes' vagal stimulation. During continued stimulation 40 cc. venous gastric blood from the first cat, withdrawn during vagus stimulation, were slowly injected into the iliac vein. The gastric secretion tended to increase and the juice became congo-positive. After injection of a further 30 cc. blood the secretion increased markedly.

This result indicated that a gastric secretory excitant might be liberated and absorbed into the blood stream during vagal

stimulation. A further support for this view was obtained from cross-circulation experiments, one of which is shown in fig. and table XIV. In this experiment the pyloric mucosa of a cat (donor) was cocainized. After this the blood supply of a second cat's stomach (recipient) was arranged in such a way that the donor's carotid artery was anastomosed with the recipient's coeliac artery and the recipient's superior mesenteric vein with the

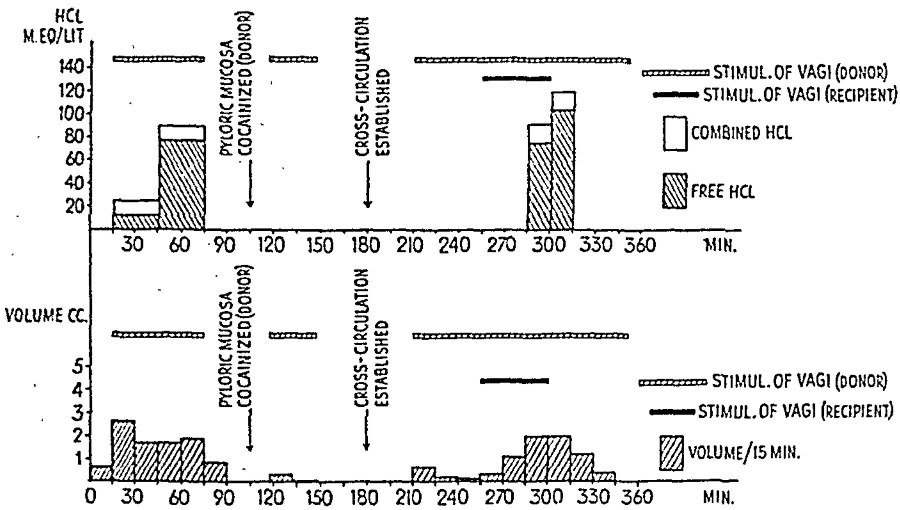

Fig. XIV. Cross-circulation experiment. Cat no. 1 (donor), cat no. 2 (recipient). Cross-circulation established after cocainization of the donor's pyloric mucosa. Gastric secretion of donor cat during vagal stimulation; during a definite period the vagi of the recipient cat were concomitantly stimulated (for details see table XIV).

donor's jugular vein. The portal vein and the hepatic artery of the recipient cat were ligated in the duodeno-hepatic ligament. Since the superior mesenteric vein was ligated distally to the inflow of the gastro-splenic vein, the venous outflow from the recipient's stomach was diverted to the donor's jugular vein. In both animals clotting was prevented by heparine.

The recipient's stomach being connected with the circulation of the donor, the vagus nerves of the latter were stimulated. During 45 minutes' stimulation only a scanty flow of congo-negative juice was secreted. When however the recipient's vagus nerves were also stimulated, the gastric secretion in the

stomach of the donor increased considerably and free acid appeared in the juice. The acidity even reached a higher level than previously. When the stimulation of the recipient's vagus nerves was stopped, the secretion dropped in spite of continued stimulation of the donor.

Six cross-circulation experiments of this type were performed. In some experiments the procedure was somewhat modified.

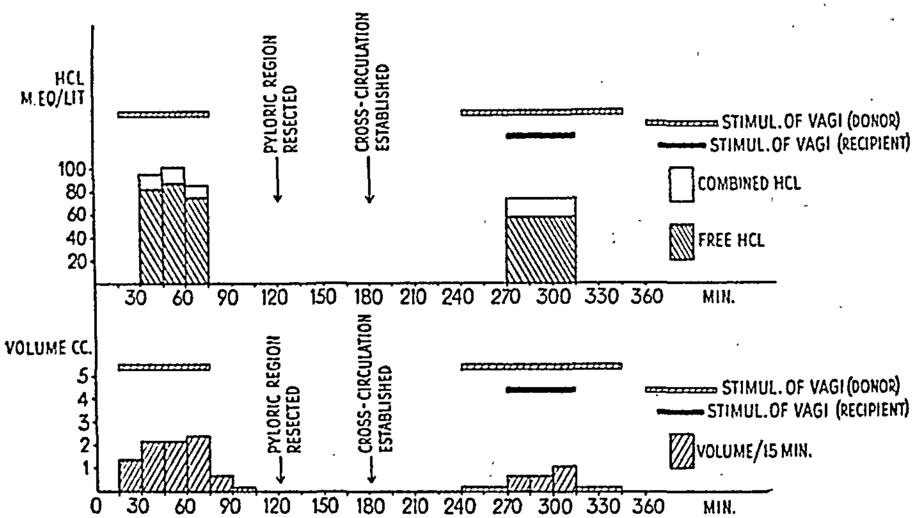

Fig. XV. Cross-circulation experiment. Cat no. 1 (donor), cat no. 2 (recipient). Cross-circulation established after resection of donor's pyloric region. Gastric secretion of donor cat during vagal stimulation; during a definite period the vagi of recipient cat were concomitantly stimulated (for details see table XV).

The recipient's venous gastric blood was collected in a vessel and slowly injected into the donor's iliac vein. By this technique the venous flow from the recipient and the inflow to the donor could be controlled, avoiding abrupt changes in the blood flow. The recipient's spleen was resected in order to obtain pure gastric blood.

In three cases the donor died before the experiment was completed. In these experiments a definite initial secretion of watery acid gastric juice was observed in the donor on stimulation of the vagus nerves of the recipient. In three other experiments, two were made after cocainization and one after

resection of the donor's pyloric region. This experiment is demonstrated in fig. and table XV. After resection of the pyloric region of the donor cat and after the establishment of cross-circulation only a few tenths of cc. were secreted during 30 minutes' vagal stimulation. During concomitant stimulation of the recipient's vagus nerves, the *secretion rose*, *to decrease* again when this stimulation was interrupted.

These experiments strongly favour the view that a gastric secretory excitant is liberated into the blood stream during vagus stimulation. Under the given experimental conditions and with the support of our previous results, the pyloric region must be regarded as the origin of this factor. The liberation into the blood of the humoral agent seems to be under control of the central nervous system, since secretion is only excited if the recipient's vagus nerves are stimulated.

In three experiments, one of which is seen in table XV, where the venous outflow from the recipient's stomach was measured, the rate of flow increased on vagal stimulation. This increase is obviously due to dilatation of the vessels of the stomach. There is no reason to believe that the increased delivery of gastric venous blood to the donor is in itself sufficient to promote secretion.

Extraction of a secretagogue agent from the pyloric mucosa.

Attempts were made to demonstrate a gastric secretory excitant in the pyloric mucosa. The tissue was extracted by using a technique devised by Komarov (1938). Gastric mucosa from cats and dogs — in a few experiments from pigs — was used.

The gastric mucosa from the pyloric or corpus region of a freshly-killed animal was excised. The mucosa was minced with scissors and boiled together with 10 volumes N/10 HCl, and partially neutralized with N NaOH so that it remained only slightly acid to congo. After cooling the mixture was centrifuged, the supernatant fluid filtered through cotton wool, and the filtrate precipiated with 10 % trichloracetic acid. The precipitate was centrifuged and washed 3 times with 40—50

volumes of 10 % trichloracetic acid in saline, twice with 50 volumes of acetone, once with benzene, and twice with ether, and dried at 37° C.

Usually the extracts were made from 5—10 gm. moist mucosa yielding 25—50 mg. dry residue. On dissolving this quantity

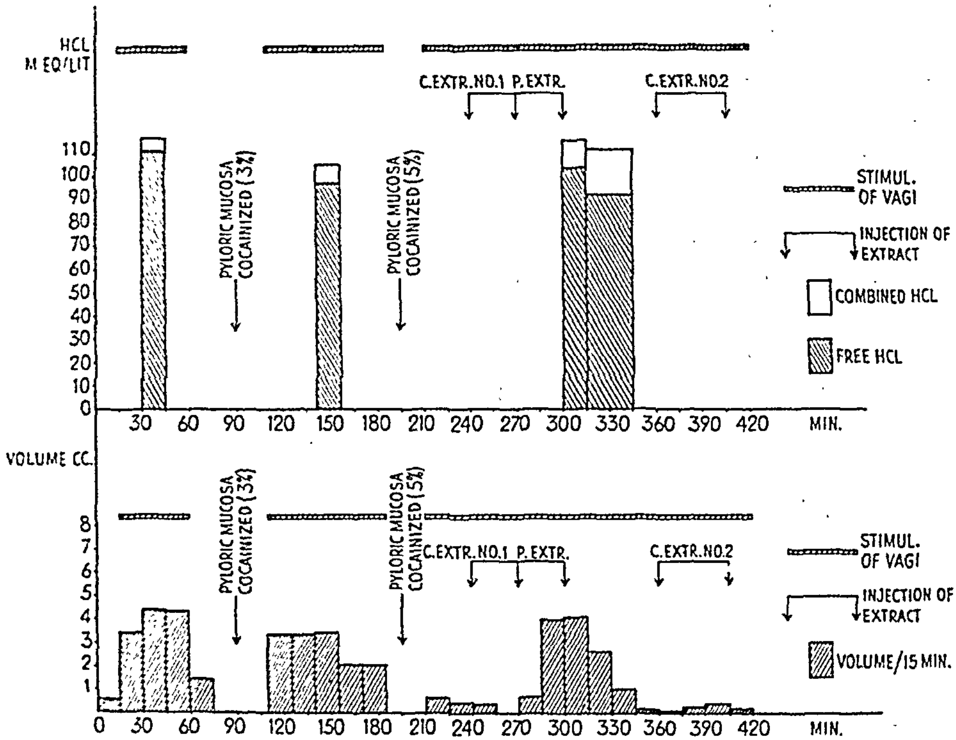

Fig. XVI. Cat. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extracts from cat's pyloric and corpus mucosa were injected intravenously; during definite periods the vagi were concomitantly stimulated (for details see table XVI).

in 20—30 cc. Tyrode solution an opalescent fluid was obtained. The extracts were slowly injected into the iliac vein at a rate of 0.4 cc. per minute.

The extracts were tested on animals with their pyloric region cocainized or resected. In tests on cats 16 extracts from cat's pyloric mucosa all showed activity. In a few experiments the secretory effect was only slight, but in the rest abundant secretion was obtained. Contrary to this, 11 extracts from cat's

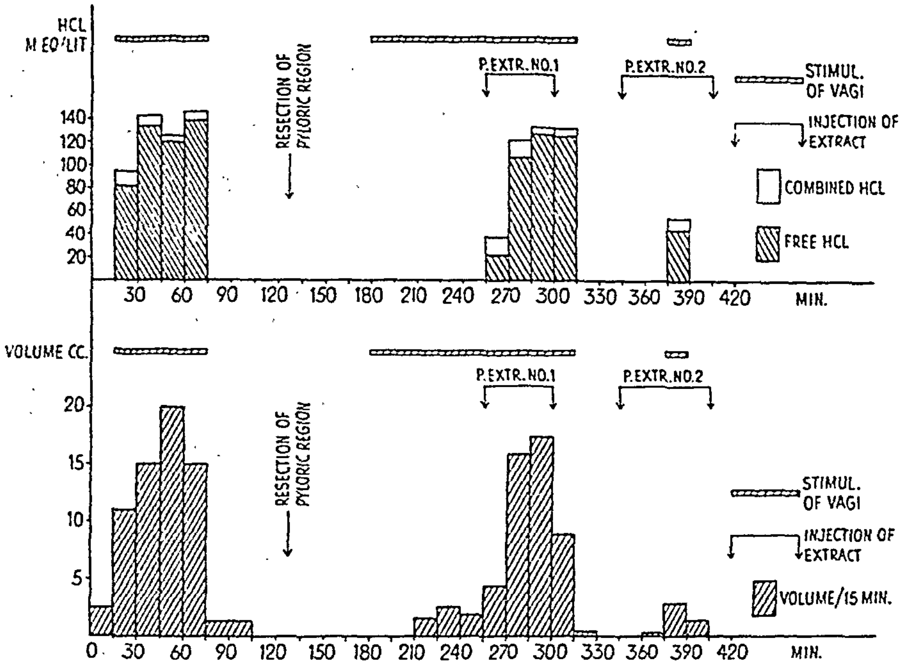

Fig. XVII. Dog. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Following this extracts from canine pyloric mucosa were injected intravenously. During definite periods the vagi were concomitantly stimulated (for details see table XVII).

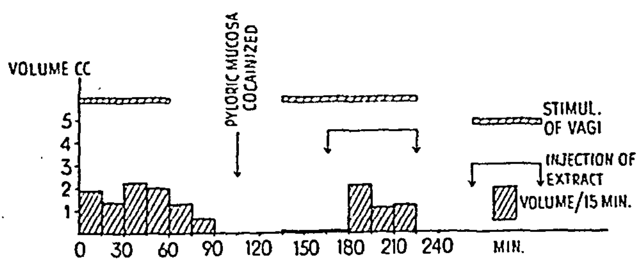

Fig. XVIII. Cat. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extracts from pig's pyloric mucosa was injected intravenously. The vagi were concomitantly stimulated (for details see table XVIII).

corpus mucosa were all inactive (with the possible exception of the corpus extract no. 2, v. fig. and table XVI). An experiment of this type is seen in fig. and table XVI. In this experiment the secretory response to vagal stimulation practically dis-

appeared after cocainization first with 3 % and then with 5 % cocaine solution. The extracts were then injected during concomitant stimulation of the vagi. The injection of an extract from pyloric mucosa (p. extr.) was followed by an abundant secretion. Two extracts from corpus mucosa (c. extr. no. 1 and 2)

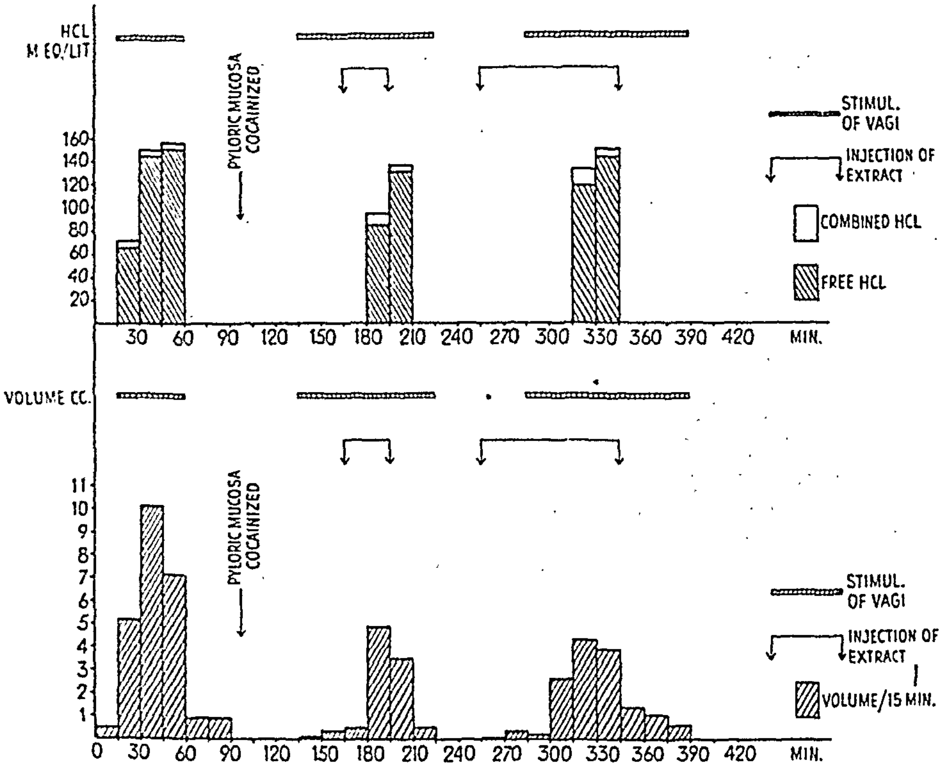

Fig. XIX. Cat. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extract from cat's pyloric mucosa was injected intravenously; during definite periods the vagi were concomitantly stimulated (for details see table XIX).

were both inactive (there may have been a very slight activity in c. extr. no. 2).

Four extracts from canine pyloric mucosa were active when tested on dogs. As shown in fig. and table XVII injection of pyloric extract (p. extr. no. 1) caused a secretion as abundant as that before the resection of the pyloric region. A second pyloric extract (p. extr. no. 2) was less active. One extract from canine pyloric mucosa was found to be active when tested

on a cat. Fig. and table XVIII show the secretory response to a pyloric extract from pig when injected into a cat.

Considering KOMAROVS report, it is not surprising that injection of pyloric extracts caused gastric secretion. The following observations, however, are on quite a different line from those of KOMAROV. In the experiment shown in fig. and table XIX the pyloric mucosa of a cat was cocainized and the vagi stimulated. After the injection of pyloric extract abundant secretion occurred. The secretion persisted circa 15 minutes after the end of the injection. It then stopped almost completely in spite of continued vagal stimulation. After 30 minutes' rest, pyloric extract was again injected without stimulation of the vagi. During 30 minutes only a few tenths of cc. were secreted. If, however, the vagi were stimulated concomitantly with the slow injection of pyloric extract, an abundant flow of gastric juice occurred.

The observation that secretion was only excited by the simultaneous influence of pyloric extract and vagal impulses was confirmed in 6 experiments on cats and 4 on dogs. One experiment on a dog is seen in fig. and table XX. In this experiment, after resection of the pyloric region, only a thick mucus was secreted during stimulation of the vagi. Infusion of pyloric extract no. 1 alone during 30 minutes caused no increase of secretion. After vagal stimulation also had been applied, however, the acidity increased and the juice became more watery. Injection during 30 minutes of a second extract (p. extr. no. 2) caused only a scanty secretion. On application of vagal stimulation the secretory rate rose quickly. In all experiments in which gastric secretion was excited by concomitant nerve stimulation and injection of extract the secretory response to vagal stimulation still remained for a time after the end of injection. The secretion generally remained abundant during the first 15 minutes, and then declined. After 45 minutes the secretion was again on the same low level as before injection.

In some experiments the injection of pyloric extracts alone caused secretion. This secretion was generally of low rate and increased strongly after application of vagal stimulation. The increase in rate of secretion was of such an order that it could

not be due simply to summation of the poor secretory effects of nerve stimulation and extract if applied separately; nor could it be due to the motor effect of vagal stimulation. This could be excluded because of the fact that in most of the experiments

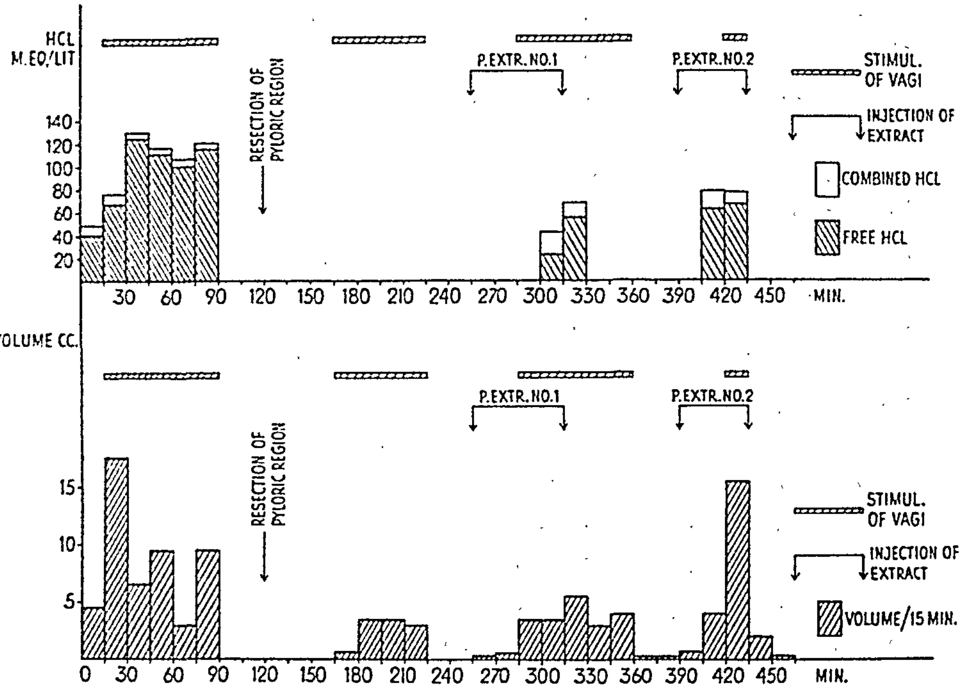

Fig. XX. Dog. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Following this, extracts from canine's pyloric mucosa were injected intravenously. During definite periods the vagi were concomitantly stimulated (for details see table XX).

there was a latency of about 15 minutes between the application of the simultaneous stimuli and the onset of the secretory response. In addition it was always controlled in these experiments that no gastric content had stagnated before the beginning of stimulation.

In two experiments on cats, a considerable secretion was observed already during the injection of pyloric extracts. In one of these experiments (fig. and table XXI) secretion rose strongly on vagal stimulation. In the second (fig. and table XXII) no obvious increase of the secretory volume was observed,

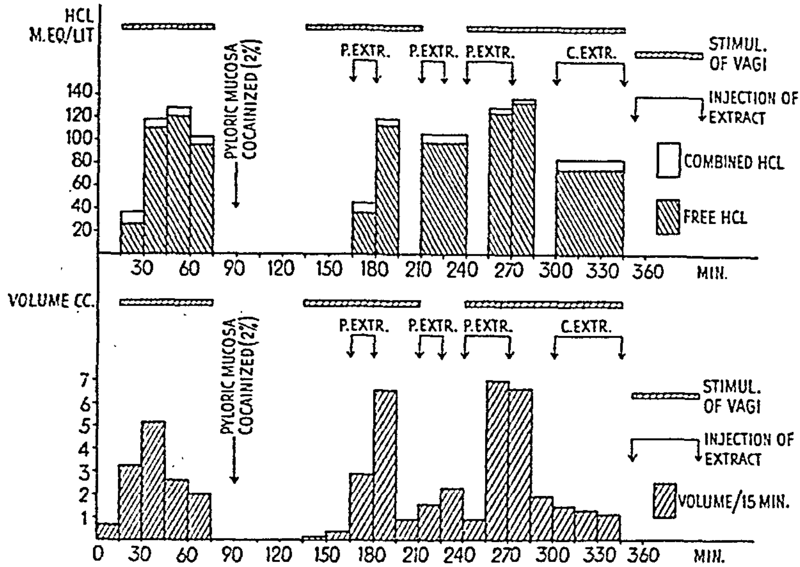

Fig. XXI. Cat. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extracts from cat's pyloric and corpus mucosa were injected intravenously; during definite periods the vagi were concomitantly stimulated (for details see table XXI).

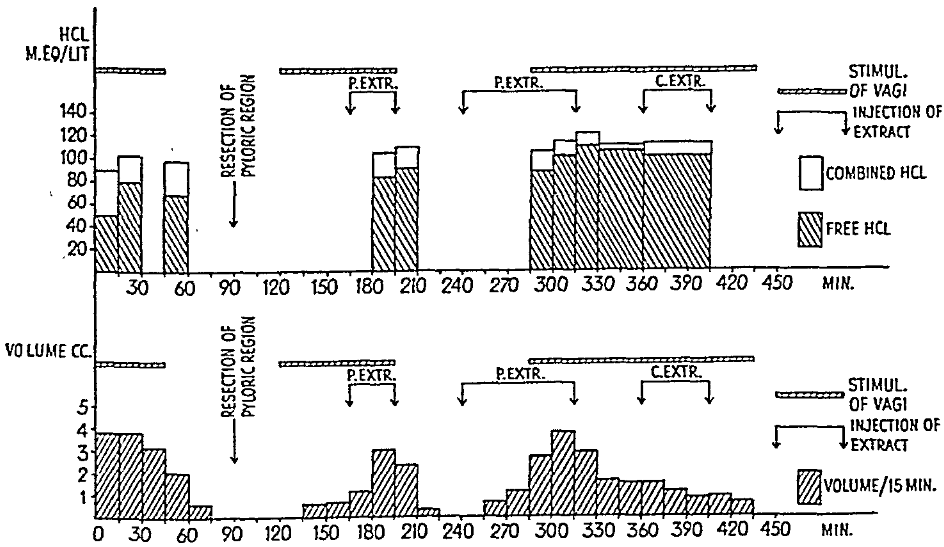

Fig. XXII. Cat. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Following this, extracts from cat's pyloric and corpus mucosa were injected intravenously; during definite periods the vagi were concomitantly stimulated (for details see table XXII).

even though under the influence of vagal stimulation secretion tended to reach its maximum quicker.

At the present time no explanation can be offered of the fact that in some experiments injection of extracts alone is sufficient to excite gastric secretion, whereas in other experiments additional vagal stimulation is necessary. A more detailed analysis of these observations has been postponed, awaiting the results of experiments now in progress to obtain the agent in the pure state. The differences in the secretory effect are possibly due to different concentrations of the active principle in the various extracts.[1] The possibility that two different active principles are concerned cannot however be rejected at this stage.

The secretagogue factor and histamine.

Komarov claims that the active principle extracted by him is histamine-free. We have estimated the histamine activity of the pyloric extracts, using the guinea pig's small intestine and the cat's blood pressure as tests.

11 pyloric extracts and 5 corpus extracts from cats and 4 pyloric extracts from dogs were tested for their histamine content. As will be seen from the table p. 45 the highest observed histamine activity in pyloric extracts corresponds to 0.12 γ histamine base per cc. When injecting 0.4 cc. pyloric extract per minute into a cat weighing 3 kg., 0.016 γ histamine per kg. and minute are administered. In our experience as well as that of others (Emmelin et *al.* 1941) this histamine dose is far below the amount necessary to excite gastric secretion. In cats 0.5—1 γ per kg. and minute causes only a scanty flow. In unanaesthetized dogs Ivy and Javois (1925) observed gastric

[1] It should be pointed out that some of the extracts showed a remarkably high activity. In the experiment shown in fig. and table XVII the quantity of dry substance (p. extr. no. 1) amounted to only about 10 mg. This was dissolved in 20 cc. Tyrode solution and injected at a rate of 0.4 cc. per min. As the dog weighed 11.5 kg., less than 20 γ crude extract per kg. body-weight and min. was injected. During this injection vagus stimulation caused a secretion as abundant as before resection of the dog's pyloric region.

secretion during intravenous injection of 0.0027 mg. per kg. and minute. In order to obtain a secretion corresponding to that which we observed on injection of active extracts, at least 5 γ histamine per kg. and minute was required. Thus the activity of the extracts cannot possibly be due to histamine, this being already evident from the fact that the inactive corpus extracts showed more histamine activity than the active pyloric extracts.

Histamine content in mucosal extracts.

Animal	Region of stomach	Histamine base γ/cc.
Cat	Pyloric mucosa	0.12
»	»	0.10
»	»	0.06
»	»	0.02
»	»	0.02
»	»	0.02
»	»	0.02
»	»	0.02
»	»	0.02
»	»	0.01
»	»	0.01
»	Corpus mucosa	0.10
»	»	0.10
»	»	0.03
»	»	0.03
»	»	0.02
Dog	Pyloric mucosa	0.08
»	»	0.04
»	»	0.03
»	»	0.03

Three pyloric extracts were tested for their histamine activity on the anaesthetized cat's blood pressure. Contrary to histamine, which caused a marked fall in blood pressure, the injection of from 0.1 to 2 cc. of pyloric extracts caused no fall in blood pressure. An experiment of this type is seen in fig. 8.

It has been shown in man and in dogs (WANGENSTEEN et *al.*

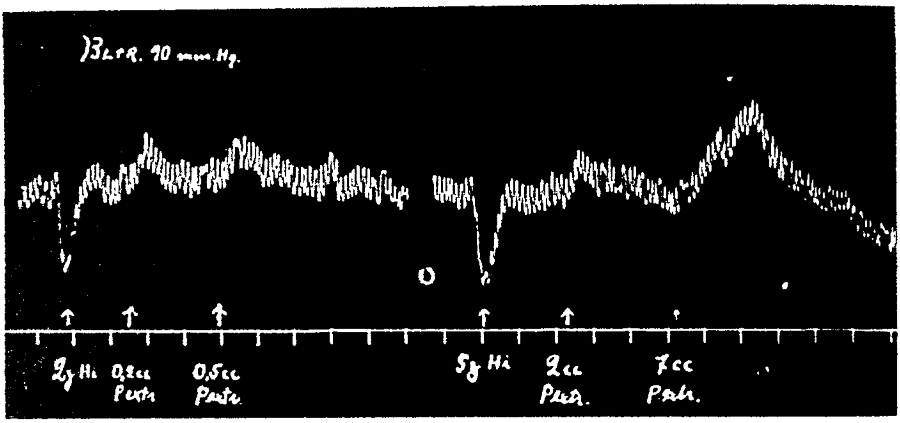

Fig. 8. Cat 3.2 kg. anaesthetized with chloralose-urethane. Blood pressure response to intravenous injection of histamine and active pyloric extract (p. extr. no. 1 as used in exp. seen in table XVII). Time in minutes.

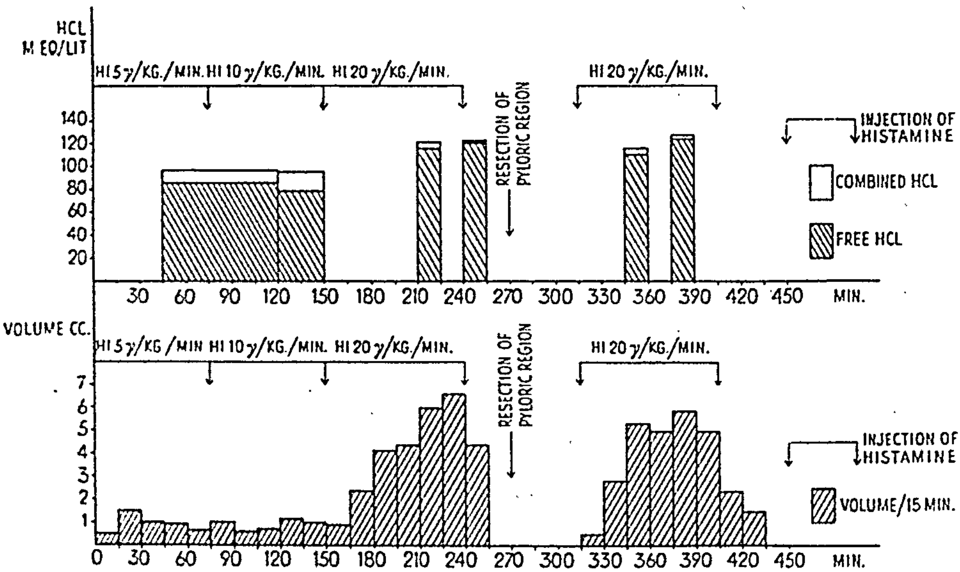

Fig. XXIII. Cat. Gastric secretion during intravenous injection of histamine before and after resection of pyloric region (for details see table XXIII).

1940 and others) that histamine excites gastric secretion even after resection of the pyloric region. WANGENSTEEN et *al.*, however, report that after extensive resections of the stomach in man histamine did not excite gastric secretion, although HCl-secreting parenchyma was still present in the stomach. In our own

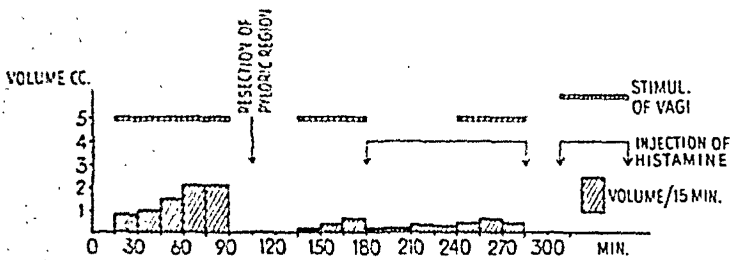

Fig. XXIV. Cat. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Histamine was slowly injected intravenously; the vagi were stimulated during later stage of injection (for details see table XXIV).

experiments cocainization or resection of the pyloric region did not alter the secretory effect of histamine (v. fig. and table XXIII).

In a series of experiments the effect of concomitant injection of histamine and vagal stimulation on animals with their pyloric region resected was investigated. If histamine was slowly injected in amounts just sufficient to cause a scanty flow of gastric secretion, the rate of flow did not increase when vagal stimulation was superimposed (v. fig. and table XXIV).

It has not been our intention in this work to study in detail the biochemical properties of the active principle in the pyloric extract. These problems will be delt with in later papers from this laboratory. The following fact has been ascertained: the activity of the dry residue from pyloric extracts does not diminish appreciably if kept in the ice box during a month; if it is dissolved in Tyrode solution of slight acid reaction and cept cold, the activity does not deteriorate within 14 days.

Discussion.

Liberation of a hormone from the pyloric region.

From the experiments described in this paper it is evident that the pyloric region plays an important part in the cephalic phase of gastric secretion. After interference with the function of this region the HCl-secretion during stimulation of the vagi decreases considerably or ceases completely. This fact, as also the demonstration of a gastric secretory excitant in extracts from pyloric mucosa but not in those from corpus mucosa, strongly favour the idea of a humoral mechanism. This conception is strongly supported by cross-circulation experiments where a secretory excitant is demonstrated in the venous flow from the stomach during vagal stimulation.

Since EDKINS (1906) presented the gastrin theory, which was modified and extended in a large number of later works, the idea has been generally accepted that the gastric phase of secretion is regulated by a combined neuro-humoral mechanism (IVY 1930). By the contact of the food with the gastric mucosa a secretory excitant is supposed to be liberated into the blood stream. The majority of investigators assume that the mucosa of the pyloric region is more important than the corpus mucosa in this process, although this idea has not been generally accepted.

Some recent investigations (LIM, IVY and MCCARTHY 1925, STEINBERG et *al.* 1927 etc.) support the idea that HCl-secretion can be excited without co-operation of the pyloric region. It must be pointed out that EDKINS already demonstrated a secretory excitant in extracts from cardiac mucosa. The importance of this region for the persistence of gastric secretion after resection of the pyloric region has not been taken into

account in later experimental works. The similar anatomical structure of the mucosa of the cardiac, pyloric and duodenal regions indicates a common function.

Although a humoral mechanism is generally accepted, there is no agreement as to whether the humoral agent is a hormone liberated from the gastric mucosa or a secretagogue derived from the food during digestion. The inherent difficulty of this problem lies in the fact that the food or digestive products introduced into the stomach may themselves stimulate the gastric glands after having been absorbed into the blood stream. There are, however, definite facts pointing to the active agent's being of hormonal nature. SAVITSCH and ZELIONY (1913) observed gastric secretion on introduction of soap- and acetic acid solutions into an isolated pyloric pouch. It is very doubtful if these substances are absorbed into the blood stream (BABKIN 1928). ZELIONY and SAVITSCH (1911/12) report that cocaine applied locally to the isolated pyloric pouch mucosa inhibited the secretory effect of food and digestive products. LIM, IVY and McCARTHY (1925) were able to excite secretion in the fundus by mechanical stimulation of the mucosa in an isolated pyloric pouch.

The humoral agent intervening in the *cephalic phase* cannot be derived from food or digestive products. All our experiments have been carried out on fasting animals with empty stomachs. It seems justifiable to conclude that the secretory excitant of the *cephalic phase* is of a hormonal nature.

The chemical nature of the pylorical hormone.

Previous attempts to isolate the gastric hormone delt mainly with protein-free extracts of gastric mucosa. The active agent in these extracts has been considered by some to be histamine (SACHS, IVY, BURGESS and VANDOLAH (1932) and others) or a substance similar to histamine (the gastrin bodies of KOCH, LUCKHARDT and KEETON). GAVIN, McHENRY and WILSON (1933) showed that the fundic mucosa contained greater quantities of histamine than the pyloric mucosa. This fact was later confirmed by other investigators. Since the gastric phase is excited mainly

via the pyloric mucosa, these facts do not favour that histamine is the gastric hormone.

In contrast to earlier investigators, KOMAROV hypothesized that the gastric hormone is a protein possibly similar to secretin. He investigated the protein fraction of the mucosal extracts and claims to have demonstrated a gastric secretory excitant.

We have confirmed this statement, since our extracts are histamine-free or of very low histamine activity. Probably the active factor is a protein. Whether it is related chemically to secretin will probably be evident from experiments proceeding in this laboratory.

The mode of action of the pyloric hormone.

Our experiments show that only in combination with vagal impulses does injection of extracts cause abundant secretion.

In the present stage of knowledge there is not much basis for an understanding of the mode of co-operation between the humoral and the nervous mechanisms. Like other workers, we have found that the blood flow through the stomach is increased on stimulation of the vagi. It is not possible to decide what part the increased blood flow plays in stimulating the gastric secretion. In various works IVY has stressed the possible importance of vaso-dilatation for the stimulation of gastric secretion. However, the active pyloric agent described in this paper has no effect on the general arterial blood pressure. Even after intravenous administration of large doses of active extracts to unanaesthetized cats, no fall in blood pressure occurs.

The humoral agents acting in the cephalic and gastric phases may be identical.

It is tempting to suppose that the humoral factors of the two secretory phases are identical. Some common features of the humoral agents support this idea.

Both humoral factors are liberated by a combined neuro-humoral mechanism.

As already mentioned, ZELIONY and SAVITSCH found that cocaine instillation into an isolated pyloric pouch stopped the secretory effect of various food substances introduced into the pouch. These authors consider this to be due to a paralysis of afferent nerve endings in the mucosa, reflex paths being interrupted. This conception was later revised by SAVITSCH (1922). According to him the liberation of a hormone was prevented by cocainization. The effect of cocaine could not be due to local vasoconstriction, since local administration of adrenalin did not inhibit the secretory response. These workers, as also LIM, IVY and MCCARTHY (1925), showed that injection of atropine inhibited the gastric secretion obtained on introduction of food into an isolated pyloric pouch. According to IVY the inhibitory effect of antropine suggests that post-ganglionic vagal structures are essential in initiating the gastric phase of secretion.

Our results indicate that the hormone liberation from the pyloric region is under central nervous vagal control. After disconnecting the pyloric region from the stomach, the secretory effect of vagus stimulation decreased considerably. By this operation the pyloric region was deprived of at least the greater part of its vagal innervation. It is likely that the reduction in secretion is due to an elimination of pre-ganglionic vagal impulses, with accompanying reduced hormonal liberation to the blood stream. The existence of a central nervous control is further evident from cross-circulation experiments. In these the humoral factor occurs in detectable amounts only when the vagi are stimulated.

According to SCHABADASCH 1930 and others in sections of the gastric wall nerve cells are found in greater amounts in pyloric region than in the corpus. It is uncertain if these nervous structures are of vagal origin. Some investigators claim that they are both vagal and sympathetic, whereas others consider it impossible to decide what is the nature of these nervous structures.

From our experiments it is likely that the obstruction of the cephalic phase of gastric secretion following cocainization of the pyloric region is due to paralysis of pyloric nervous struc-

tures. It is further likely that the functional integrity of these nervous structures is a necessity for the liberation of the humoral factors intervening in the gastric and the cephalic phases of secretion.

Since the humoral factors engaged in the cephalic and gastric phases of secretion are both liberated from the pyloric region, and since both are probably liberated by the activity of local nervous structures in this region, it is tempting to assume that the humoral factors engaged in the two phases are identical.

Ivy, Lim and MacCarthy (1925 b), working in dogs with entire stomach pouches and direct connection between oesophagus and duodenum, observed gastric secretion in the pouch after oral administration even of unabsorbable agencies such as saponines. Komarov (1938) claims to have extracted from the dogs duodenal mucosa a gastric secretory excitant other than histamine. These observations suggest that the intestinal phase is initiated by a humoral factor liberated from the intestinal mucosa.

It may be hypothesized that all three phases of gastric secretion are excited by a similar mechanism and that one and the same humoral factor is engaged in the three phases.

Is the conception of a neuro-humoral mechanism in the cephalic phase in harmony with previous observations?

It is generally assumed that the cephalic and gastric phases of secretion are initiated in fundamentally different ways, the former by impulses conveyed by the vagi to the secretory cells, the latter by a combined neuro-humoral mechanism. According to the conception put forward in this work, both phases are initiated by fundamentally similar mechanisms, both neuro-humoral in nature.

According to older clinical reports the psychic secretion is strongly reduced after resection of the pyloric region. This seemed surprising and could not be explained by the prevailing conception. Schur and Plaschkes (1915) for example comment: »Es ist eigentlich nicht ganz verständlich, dass die Entfernung des pylorischen Abschnittes zur vollkommener Anazidität des Mageninhaltes führt, auch wenn die Nahrung per os darge-

reicht wird.» Similar conclusions are drawn by GOETZE (1927), LITTHAUER (1920) and others. As far as human pathology is concerned there seem to exist no recent papers on the psychic gastric secretion following resection of the pyloric region. Modern techniques of fractional testing of the gastric juice have been employed in studying the gastric secretion after gastric resections. A persistent HCl-secretion has been demonstrated in a large percentage of the cases. The persistent HCl-secretion has been considered by a number of workers to indicate that the psychic phase of secretion has not been interfered with by the operation. Applying the Rehfuss test meal, KLEIN (1927) observed a considerable decreased HCl-secretion after resection of the pyloric region; chewing a slice of lemon, however, appreciably increased the secretion. This again was taken as evidence that the psychic phase was not influenced by resection of the pyloric region.

It should be noted that in clinical investigations of more recent years histamine and alcohol have been used as secretory stimulants. As has long been known, these substances stimulate the HCl-secreting glands directly without co-operation of nervous structures. These investigations therefore give no information about the mechanisms instrumental in the psychic phase.

In experiments on animals a considerable secretion has been found to persist after resection of the pyloric region. PORTIS and PORTIS (1926), SMIDT (1923), STEINBERG et *al.* (1927) etc. observed secretion in the Pavlov pouch of dogs after such operations. They conclude that the psychic phase persists after resection of the pyloric region. However, these authors have not studied the two phases separately.

In all these experiments secretion was excited by test meals. As far as we know, the only work studying the psychic phase separately was carried out by STRAATEN (1933), who reports that the gastric secretion excited by sham feeding of dogs decreased after resection of the pyloric region. In his experiments pronounced hyposecretion persisted 6—8 months after pyloric resection. After only partial resection of the pyloric

region, secretion tended to increase again during the course of some months.

The fact that the psychic phase may in some instances persist after resection of the pyloric region may be due to liberation of the hormone from some other source. It is conceivable that the hormone may be derived from the duodenal mucosa in sufficient amounts to make the HCl-glands respond to vagal impulses.

Summary.

The factors controlling the cephalic phase of secretion have been analyzed in dogs and cats.

Evidence is presented in favour of the view that the cephalic phase of gastric secretion is controlled by a combined neuro-humoral mechanism identical in principle with that of the gastric phase. The pyloric region plays a major part in this mechanism.

The main results supporting this view are the following: —

I. The gastric secretion during vagal stimulation stops or is greatly diminished

a) if the pyloric region is deprived of its arterial blood supply.

b) if the venous blood flow from the pyloric region is obstructed.

c) if the function of the pyloric mucosa is depressed by cocainization.

d) if the pyloric region is resected.

II. A secretagogue agent is liberated from the pyloric region into the blood stream during vagus stimulation, as shown in cross-circulation experiments.

III. A secretagogue factor can be extracted from pyloric mucosa of cats and dogs. The extracts have been tested on animals with their pyloric region cocainized or resected. These tests indicate that

a) by intravenous injection of the extract alone only a very slight secretion follows.

b) by intravenous injection of the extract during concomitant stimulation of the vagi an abundant secretion occurs.

The extracted mucosal factor is not histamine.

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TABLE I.

Cat 3.7 kg. Gastric secretion during stimulation of vagi. Intensity of stimulus 1.5 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Stimul. of vagi	10.30—10.45	15	7.6	13.5	37.0
2.	»	10.45—11.00	15	5.0	110.5	126.0
3.	»	11.00—11.15	15	5.4	153.0	161.0
4.	»	11.15—11.30	15	4.2	136.0	159.0
5.	»	11.30—11.45	15	4.4	143.0	155.5
6.	»	11.45—12.00	15	4.3		
7.	»	12.00—12.15	15	6.1		
8.	»	12.15—12.30	15	5.5	152.0	158.0
9.	»	12.30—12.45	15	3.9		
10.	»	12.45—13.00	15	5.0	127.5	149.5
11.	»	13.00—13.15	15	2.9		
12.	»	13.15—13.30	15	3.2	151.0	156.5
13.	»	13.30—13.45	15	2.7		
14.	»	13.45—14.00	15	2.6	116.0	132.0
15.	»	14.00—14.15	15	2.4		
16.	»	14.15—14.30	15	2.5	94.5	105.0
17.	»	14.30—14.45	15	2.5		
18.	»	14.45—15.00	15	2.5	96.0	102.5
19.	»	15.00—15.15	15	2.5		

TABLE II.

Cat 3.5 kg. Gastric secretion during stimulation of vagi before and after ligature around pylorus and adjacent arteries. Intensity of stimulus 1 m. a.

Sample	Procedure	Time hour	Time min.	Gastric Secretion volume cc
1.	Stimul. of vagi	11.00—11.15	15	5.0
2.	»	11.15—11.30	15	4.5
3.	»	11.30—11.45	15	4.5
4.	»	11.45—12.00	15	5.5
5.	»	12.00—12.15	15	3.1
6.	»	12.15—12.30	15	2.7
7.	»	12.30—12.45	15	3.0
8.	»	12.45—13.00	15	3.1
	Ligature around pylorus and adjacent arteries	13.00—13.30	30	
9.	Stimul. of vagi	13.30—13.45	15	0.5
10.	»	13.45—14.00	15	0.2
11.	»	14.00—14.15	15	0.1
12.	»	14.15—14.30	15	a few drops
13.	»	14.30—14.45	15	»
14.	»	14.45—15.00	15	»
15.	»	15.00—15.15	15	»
16.	»	15.15—15.30	15	»

TABLE III.

Cat 2.4 kg. Gastric secretion during stimulation of vagi before and after ligature around pylorus and adjacent arteries. At a later stage the ligature was removed and following this, only the adjacent veins were ligated. Intensity of stimulus 1 m. a.

Sample	Procedure	Time hour	Time min.	Gastric Secretion volume cc.	Gastric Secretion pH	Gastric Secretion character of juice
1.	Gastric content	11.25		3.2	4.3	watery
2.	No stimulation	11.25—12.10	45	2.5	5.5	»
3.	Stimul. of vagi	12.10—12.40	30	7.3	1.3	»
4.	»	12.40—13.10	30	10.5	1.2	»
5.	No stimulation	13.10—13.25	15	0.5		»
	Ligature around pylorus and adjacent vessels	13.25—13.55	30			
6.	Stimul. of vagi	13.55—14.10	15	1.0		mucous
7.	»	14.10—14.25	15	0.2		»
8.	»	14.25—14.40	15	0		»
9.	»	14.40—14.55	15	0.2		»
	Ligature opened	14.55—15.10	15			
10.	Stimul. of vagi	15.10—15.40	30	6.8	1.3	vatery
11.	»	15.40—16.10	30	6.8	1.2	»
	Ligation of veins	16.10—16.25	15			
12.	Stimul. of vagi	16.25—16.40	15	2.0		mucous
13.	»	16.40—16.55	15	0.7	4.6	»
14.	»	16.55—17.10	15	0.9		»
15.	»	17.10—17.25	15	0.5		»
16.	»	17.25—17.55	30	1.3	2.7	»
17.	»	17.55—18.25	30	1.5		less mucous

TABLE IV.

Cat 3.7 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after ligation of arteries to pyloric region. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	pH	character of juice
1.	Gastric content	9.50		4.0	2.3	watery
2.	No stimulation	9.50—10.05	15	0.6		
3.	Stimul. of vagi	10.05—10.20	15	5.5	1.7	»
4.	»	10.20—10.35	15	10.0	1.2	»
5.	»	10.35—10.50	15	12.6	1.1	»
6.	»	10.50—11.05	15	9.4	1.1	»
	Ligation of arteries to pyloric region	11.05—11.20	15			
7.	No stimulation	11.20—11.35	15	0		
8.	Stimul. of vagi	11.35—11.50	15	1.2	8.4	stringy, mucous
9.	»	11.50—12.05	15	1.9	1.5	»
10.	»	12.05—12.20	15	2.6	1.4	less mucous
11.	»	12.20—12.35	15	2.1	1.3	»

TABLE V.

Cat 3.4 kg. Gastric secretion during stimulation of vagi before and after ligation of veins from pyloric region. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Gastric content	11.20		3.0		
2.	No stimulation	11.20—11.35	15	1.1		
3.	»	11.35—11.50	15	0.6		
4.	»	11.50—12.05	15	0.1		
5.	Stimul. of vagi	12.05—13.05	60	10.3	88.0	94.0
6.	»	13.05—14.05	60	8.9	66.5	84.0
	Pyloric veins obstructed	14.05—14.20	15			
7.	Stimul. of vagi	14.20—15.20	60	3.1	39.0	45.0
	Pyloric veins open	15.20—15.35	15			
8.	Stimul. of vagi	15.35—16.35	60	7.5	100.5	111.5

TABLE VI.

Cat 3.1 kg. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	character of juice	intensity of stimulation
1.	No stimulation	13.10—13.25	15	0.1		
2.	»	13.25—13.40	15	0.5		
3.	Stimul. of vagi	13.40—13.55	15	6.5	watery	0.5 m. a.
4.	»	13.55—14.10	15	2.8	»	»
5.	»	14.10—14.25	15	2.0	»	»
6.	»	14.25—14.40	15	4.5	»	»
	Pyloric mucosa cocainized	14.40—15.25	45			
7.	Stimul. of vagi	15.25—15.40	15	0.1	mucous	1.5 m. a.
8.	»	15.40—15.55	15	0	»	2.0 m. a.
9.	»	15.55—16.10	15	1 drop	»	»
10.	»	16.10—16.25	15	»	»	»
11.	»	16.25—16.40	15	»	»	»
12.	»	16.40—17.30	50	a few drops	»	0.5 m. a.
13.	»	17.30—19.00	90	0.3	»	»
14.	»	19.00—21.00	120	9.0	watery	»
15.	»	21.00—21.15	15	3.3	»	»

TABLE VII.

Cat 4.5 kg. Gastric secretion during stimulation of vagi before and after injection of cocaine intramuscularly. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Gastric content	10.55		3.1	10.0	20.0
2.	No stimulation	10.55—11.10	15	1.1	0	
3.	Stimul. of vagi	11.10—11.25	15	2.5	14.5	24.0
4.	»	11.25—11.40	15	4.4		
5.	»	11.40—11.55	15	5.0	121.0	126.5
	Cocaine 30 mg. intramuscularly	11.55				
6.	Stimul. of vagi	11.55—12.10	15	2.7	121.0	126.5
7.	»	12.10—12.25	15	5.7		
8.	» (respiration impaired)	12.25—12.40	15	2.6 }	87.5	94.5
9.	»	12.40—12.55	15	1.4 }		
	Artificial respiration	12.55				
10.	Stimul. of vagi	12.55—13.10	15	4.0	79.0	87.5
11.	»	13.10—13,25	15	3.1	93.5	102.0
	Cocaine 60 mg. intramuscularly	13.25				
12.	Stimul. of vagi (extreme tachycardia, clonic fits)	13.25—13.40	15	0.9 }	16.5	48.5
13.	Stimul. of vagi	13.40—13.55	15	0.9 }		
14.	»	13.55—14.10	15	0.5 }		
15.	»	14.10—14.25	15	0.4 }		

TABLE VIII.

Cat 4.1 kg. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Intensity of stimulus 1.5 m. a.

Sample	Procedure	Time		Gastric Secretion	
		hour	min.	volume cc.	acidity
1.	Gastric content	10.15		6.8	congo pos.
2.	No stimulation	10.15—10.30	15	a few drops	»
3.	Stimul. of vagi	10.30—10.45	15	1.5	congo faintly pos.
4.	»	10.45—11.00	15	2.7	congo pos.
5.	»	11.00—11.15	15	2.5	»
6.	»	11.15—11.30	15	3.1	»
7.	No stimulation	11.30—11.45	15	1.7	»
8.	»	11.45—12.00	15	0	
9.	Stimul. of vagi	12.00—12.15	15	0.6	congo neg.
10.	»	12.15—12.30	15	3.7	congo pos.
11.	»	12.30—12.45	15	3.1	»
12.	No stimulation	12.45—13.00	15	1.1	»
	Resection of pyloric region	13.00—13.30	30	0	
13.	No stimulation	13.30—14.00	30	0	
14.	Stimul. of vagi	14.00—14.15	15	0.4	congo faintly pos.
15.	»	14.15—14.30	15	0.6	congo pos.
16.	»	14.30—14.45	15	0.4	»
17.	»	14.45—15.00	15	0.3	»
18.	No stimulation	15.00—15.15	15	0.4	»
19.	Stimul. of vagi	15.15—15.30	15	0.4	»
20.	»	15.30—15.45	15	0.4	»
21.	»	15.45—16.00	15	0.5	»
22.	»	16.00—16.15	15	0.4	»
23.	»	16.15—16.30	15	0.5	»
24.	»	16.30—16.45	15	0.6	»

TABLE IX.

Dog 18.0 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Intensity of stimulus 3 m. a.

Sample	Procedure	Time: hour	Time: min.	Gastric Secretion: volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.	character of juice
1.	Gastric content	9.30		39.0	31.0	42.0	watery
2.	No stimulation	9.30— 9.45	15	4.6	14.0	28.5	»
3.	»	9.45—10.00	15	0.5			
4.	Stimul. of vagi	10.00—10.15	15	24.0	47.0	56.5	»
5.	»	10.15—10.30	15	12.5	62.5	73.5	»
6.	»	10.30—10.45	15	4.5			»
7.	»	10.45—11.00	15	16.0	56.0	64.0	»
8.	»	11.00—11.15	15	16.0			»
9.	»	11.15—11.30	15	10.5	56.5	62.5	»
10.	»	11.30—11.45	15	9.5			»
11.	»	11.45—12.00	15	13.5	46.0	64.0	»
	Resection of pyloric region	12.00—12.30	30				
12.	No stimulation	12.30—13.00	30	0			
13.	Stimul. of vagi	13.00—13.15	15	1.0	congo neg.		stringy, mucous
14.	»	13.15—13.30	15	1.8	»		»
15.	»	13.30—13.45	15	1.6	»		»
16.	»	13.45—14.00	15	1.0	»		»
17.	»	14.00—14.15	15	1.3	»		»
18.	»	14.15—14.30	15	1.5	»	25.0	»
19.	»	14.30—14.45	15	2.8	»		»
20.	»	14.45—15.00	15	2.0	»		»
21.	»	15.00—15.15	15	1.5	»		»
22.	»	15.15—15.30	15	1.5	»		»
23.	»	15.30—15.45	15	1.5	»		»

TABLE X.

Cat 3.4 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after disconnecting pyloric region from stomach. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion	
		hour	min.	volume cc.	acidity
1.	Gastric content	10.35		1.1	congo faintly pos.
2.	No stimulation	10.35—10.50	15	2.0	»
3.	»	10.50—11.05	15	0.6	»
4.	Stimul. of vagi	11.05—11.20	15	0.7	congo neg.
5.	»	11.20—11.35	15	0.9	congo pos.
6.	»	11.35—11.50	15	2.0	»
7.	»	11.50—12.05	15	1.2	»
8.	»	12.05—12.20	15	3.8	»
9.	»	12.20—12.35	15	3.8	»
10.	»	12.35—12.50	15	7.4	»
	Pyloric region disconnected from stomach	12.50 —13.20	30		
11.	No stimulation	13.20—13.35	15	0.8	»
12.	»	13.35—13.50	15	0.2	»
13.	Stimul. of vagi	13.50—14.05	15	0.2	congo neg.
14.	»	14.05—14.20	15	2.0	congo faintly pos.
15.	»	14.20—14.35	15	2.3	congo pos.
16.	»	14.35—14.50	15	2.0	»
17.	»	14.50—15.05	15	1.9	»
18.	»	15.05—15.20	15	2.4	»
19.	»	15.20—15.35	15	2.5	»
20.	»	15.35—15.50	15	3.0	»

TABLE XI.

Cat 3.6 kg. Gastric secretion during stimulation of vagi before and after disconnecting pyloric region from stomach. At a later stage the pyloric mucosa was cocainized. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion			
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.	character of juice
1.	Gastric content	11.40		3.3	37.0	50.0	watery
2.	No stimulation	11.40—11.55	15	0.3	congo pos.		»
3.	Stimul. of vagi	11.55—12.10	15	4.0	65.5	76.0	»
4.	»	12.10—12.25	15	5.5	117.5	127.0	»
5.	»	12.25—12.40	15	3.2	124.0	132.0	»
6.	»	12.40—12.55	15	2.9	124.0	132.0	»
	Pyloric region disconnected from stomach	12.55—13.15	20				
7.	No stimulation	13.15—13.30	15	0.8	congo pos.		mucous
8.	Stimul. of vagi	13.30—13.45	15	0.5	»		»
9.	»	13.45—14.00	15	3.5	80.0	96.0	»
10.	»	14.00—14.15	15	3.1	133.0	146.0	less mucous
	Pyloric mucosa cocainized (4 %)	14.15—14.30	15				
11.	No stimulation	14.30—14.45	15	0			
12.	Stimul. of vagi	14.45—15.00	15	0			
13.	»	15.00—15.15	15	2 drops	congo pos.		mucous
14.	»	15.15—15.30	15	0			
15.	»	15.30—15.45	15	0.2	»		»

TABLE XII.

Dog 15 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after disconnecting pyloric region from stomach. Intensity of stimulus 3 m.a.

Sample	Procedure	Time: hour	Time: min.	Gastric Secretion: volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.	character of juice
1.	Gastric content	12.25		4.0	0		mucous
2.	No stimulation	12.25—12.40	15	0			
3.	Stimul. of vagi	12.40—12.55	15	3.5	56.0	90.0	watery
4.	»	12.55—13.10	15	9.0	106.5	124.0	»
5.	»	13.10—13.25	15	22.0	120.0	129.0	»
6.	»	13.25—13.40	15	27.0	129.0	134.5	»
7.	»	13.40—13.55	15	23.0	133.5	139.5	»
8.	»	13.55—14.10	15	21.0	117.5	122.5	»
9.	No stimulation	14.10—14.25	15	1.5	congo pos.		»
	Pyloric region disconnected from stomach	14.25—15.10	45				
10.	No stimulation	15.10—15.25	15	1.5			
11.	»	15.25—15.40	15	0.4			
12.	Stimul. of vagi	15.40—15.55	15	3.6	5.5	23.5	stringy, mucous
13.	»	15.55—16.10	15	3.2	0		»
14.	»	16.10—16.25	15	2.8	31.5	48.5	less mucous
15.	»	16.25—16.40	15	10.0	73.5	85.0	almost watery
16.	»	16.40—16.55	15	7.5	98.0	106.5	»

TABLE XIII.

Cat no. 1 2.5 kg. Pyloric mucosa was cocainized. Gastric secretion during stimulation of vagi and intravenous injection of venous blood from stomach of cat no. 2. Following this, extract from cat's pyloric mucosa was injected intravenously. Conc. of extract: 0.3 gm. moist mucosa/cc.

Cat no. 2 3.7 kg. Venous blood was drawn from stomach during stimulation of vagi. Intensity of stimulus 1 m. a.

Sample	Procedure		Time		Gastric Secretion		
					volume cc.		acidity
	cat no. 1	cat no. 2	hour	min.	cat no. 1	cat no. 2	cat no. 1
	Pyloric mucosa cocainized (5 %)						
1.	No stimulation	Stimul. of vagi	11.45—12.15	30			
2.	Stimul. of vagi	»	12.15—12.45	30		2.5	
3.	»	Venous blood drawn during stimul. of vagi	12.45—13.00	15	0	5.0	
4.	»	»	13.00—13.15	15	0	0	
5.	»	»	13.15—13.30	15	0.2	0	congo neg.
6.	Injection of 40 cc. venous blood from cat no. 2 + stimul. of vagi		13.30—13.45	15	0.1	0	»
7.	Stimul. of vagi		13.45—14.00	15	0.4		congo pos.
8.	Injection of 30 cc. venous blood from cat no. 2 + stimul. of vagi		14.00—14.15	15	0.5		»
9.	Stimul. of vagi		14.15—14.30	15	1.2		»
10.	»		14.30—14.45	15	1.2		»
11.	»		14.45—15.00	15	0.8		»
12.	Injection of p. extr. + stimul. of vagi		15.00—15.15	15	0.8		»
13.	»		15.15—15.30	15	1.3		»
14.	Stimul. of vagi		15.30—15.45	15	1.7		»
15.	»		15.45—16.00	15	1.1		»
16.	»		16.00—16.15	15	1.2		»
			16.15—16.30	15	1.1		»

TABLE XIV.

Cross-circulation experiment. Cat. I (donor) 2.6 kg. Cat. II (recipient) 5.0 kg.
Cross-circulation established after cocainization of the donor's pyloric mucosa. Gastric secration of donor cat during vagal stimulation; during a definite period the vagi of the recipient cat were concomitantly stimulated.
Intensity of stimulus 1 m. a.

Sample	Procedure		Time		Gastric Secretion		
	cat I (donor)	cat II (recipient)	hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	No stimulation		11.30—11.45	15	0.7		
2.	Stimul. of vagi		11.45—12.00	15	2.6 }	12.0	26.0
3.	»		12.00—12.15	15	1.7 }		
4.	»		12.15—12.30	15	1.7 }	78.5	90.0
5.	»		12.30—12.45	15	1.9 }		
6.	No stimulation		12.45—13.00	15	0.9	congo pos.	
	Pyloric mucosa cocainized. Respiratory paralysis. Artificial resp.		13.00—13.25	25			
7.	Stimul. of vagi		13.25—13.40	15	0.3	congo neg.	
8.	»		13.40—13.55	15	a few drops	»	
	Cross-circulation established		13.55—15.00	65			
9.	Stimul. of vagi		15.00—15.15	15	0.7	»	
10.	»		15.15—15.30	15	0.2	»	
11.	»		15.30—15.45	15	0.1	»	
12.	»	Stimul. of vagi	15.45—16.00	15	0.3	congo faintly pos.	
13.	»	»	16.00—16.15	15	1.1	congo pos.	
14.	»	»	16.15—16.30	15	2.0	76.0	92.0
15.	»	No stimulation	16.30—16.45	15	2.0	104.0	120.0
16.	»	»	16.45—17.00	15	1.2	congo pos.	
17.	»	»	17.00—17.15	15	0.4	»	

TABLE XV.

Cross-circulation experiment. Cat I (donor) 2.8 kg. Cat II (recipient) 4.7 kg.
Cross-circulation established after resection of donor's pyloric region. Gastric secretion of donor cat during vagal stimulation; during a definite period the vagi of recipient cat were concomitantly stimulated.
Intensity of stimulus 1 m. a.

Sample	Procedure		Time		Gastric Secretion		
	cat I (donor)	cat II (recipient)	hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Gastric content		10.45		4.9	50.5	66.5
2.	No stimulation		10.45—11.00	15	1 drop	congo pos.	
3.	Stimul. of vagi		11.00—11.15	15	1.3	»	
4.	»		11.15—11.30	15	2.2	82.0	96.0
5.	»		11.30—11.45	15	2.2	88.0	101.0
6.	»		11.45—12.00	15	2.4	75.0	86.5
7.	No stimulation		12.00—12.15	15	0.7	congo pos.	
8.	»		12.15—12.45	30	0.2	»	
	Pyloric region resected		12.45—13.15	30	0		
9.	No stimulation		13.15—14.00	45	a few drops	»	
	Cross-circulation established		15.00—16.00	60			
10.	Stimul. of vagi		15.00—15.15	15	0.2	congo pos.	
11.	»		15.15—15.30	15	0.2	»	
12.	»	Stimul. of vagi	15.30—15.45	15	0.6 }	»	
13.	»	»	15.45—16.00	15	0.6 }	58.5	73.5
14.	»	»	16.00—16.15	15	1.1 }		
15.	»	No stimulation	16.15—16.30	15	0.2	congo pos.	
16.	»	»	16.30—16.45	15	0.2	»	

Venous blood flow from recipient's stomach

15.00—15.15	25 cc.	15.45—16.00	55 cc.	16.15—16.30	30 cc.
15.15—15.30	25 cc.	16.00—16.15	40 cc.	16.30—16.45	30 cc.
15.30—15.45	85 cc.				

TABLE XVI.

Cat 4.7 kg. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extracts from cat's pyloric and corpus mucosa were injected intravenously; during definite periods the vagi were concomitantly simulated.

Conc. of extracts (gm. moist mucosa/cc.): c. extr. no. 1 0.5 gm./cc.; c. extr. no. 2 1 gm./cc. and p. extr. 0.2 gm./cc.
Histamine content: c. extr. no. 1 0.1 γ/cc., c. extr. no. 2 0.1 γ/cc., p. extr. 0.02 γ/cc.
Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Gastric content	11.05		4.4	83.0	92.0
2.	No stimulation	11.05—11.20	15	0.5	congo pos.	
3.	Stimul. of vagi	11.20—11.35	15	3.4	»	
4.	»	11.35—11.50	15	4.4	110.5	116.5
5.	»	11.50—12.05	15	4.3	congo pos.	
6.	No stimulation	12.05—12.20	15	1.4	»	
	Pyloric region cocainized (3 %)	12.20—12.35	15			
7.	No stimulation	12.35—12.55	20	0		
8.	Stimul. of vagi	12.55—13.10	15	3.3	congo pos.	
9.	»	13.10—13.25	15	3.3	»	
10.	»	13.25—13.40	15	3.4	97.0	105.0
11.	»	13.40—13.55	15	2.0 }	congo pos.	
12.	»	13.55—14.10	15	2.0 }	»	
	Pyloric region cocainized (5 %)	14.10—14.20	10	0		
13.	No stimulation (artificial respiration)	14.20—14.35	15	0		
14.	Stimul. of vagi	14.35—14.50	15	0.6	congo pos.	
15.	»	14.50—15.05	15	0.4	»	

Table XVI (Cont.).

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
16.	Injection of c. extr. no. 1 + stimul. of vagi	15.05—15.20	15	0.3	congo pos.	
17.	»	15.20—15.35	15	1 drop	»	
18.	Injection of p. extr. + stimul. of vagi	15.35—15.50	15	0.7	»	
19.	»	15.50—16.05	15	4.0	»	
20.	Stimul. of vagi	16.05—16.20	15	4.1	103.0	115.0
21.	»	16.20—16.35	15	2.7 }		
22.	»	16.35—16.50	15	1.0 }	92.0	110.5
23.	»	16.50—17.05	15	0.1	congo pos.	
24.	Injection of c. extr. no. 2 + stimul. of vagi	17.05—17.20	15	2 drops	»	
25.	»	17.20—17.35	15	0.3	»	
26.	»	17.35—17.50	15	0.4	»	
27.	Stimul. of vagi	17.50—18.05	15	0.2	»	

TABLE XVII.

Dog 11.5 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Following this, extracts from canine pyloric mucosa were injected intravenously. During definite periods the vagi were concomitantly stimulated.

Conc. of extracts (gm. moist mucosa/cc.): p. extr. no. 1 0.3 gm./cc.; p. extr. no. 2 0.4 gm./cc. Histamine content: p. extr. no. 1 0.03 γ/cc.; p. extr. no. 2 0.04 γ/cc.

Intensity of stimulus 3 m. a.

Sample	Procedure	Time		Gastric Secretion			
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.	character of juice
1.	Gastric content	11.05		35.0	23.0	34.5	watery
2.	No stimulation	11.05—11.20	15	2.5			»
3.	Stimul. of vagi	11.20—11.35	15	11.0	81.0	93.0	»
4.	»	11.35—11.50	15	15.0	132.5	142.0	»
5.	»	11.50—12.05	15	20.0	120.0	125.0	»
6.	»	12.05—12.20	15	15.0	139.5	144.5	»
7.	No stimulation	12.20—12.50	30	2.5	congo pos.		»
	Resection of pyloric region	12.50—13.35	45				
8.	No stimulation	13.35—14.05	30	0			
9.	Stimul. of vagi	14.05—14.20	15	0			
10.	»	14.20—14.35	15	0			
11.	»	14.35—14.50	15	1.7	congo neg.		stringy, mucous
12.	»	14.50—15.05	15	2.7	»	pH 6.7	»
13.	»	15.05—15.20	15	2.0	»		»

Table XVII (Cont.).

Sample	Procedure	Time		Gastric Secretion			
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.	character of juice
14.	Injection of p. extr. no. 1 + stimul. of vagi	15.20—15.35	15	4.5	22.5	38.0	less mucous watery
15.	»	15.35—15.50	15	16.0	108.5	121.5	
16.	»	15.50—16.05	15	17.5	127.5	132.5	
17.	Stimul. of vagi	16.05—16.20	15	9.0	126.5	132.0	»
18.	No stimulation	16.20—16.35	15	0.5	congo pos.		»
19.	»	16.35—16.50	15	0			»
20.	Injection of p. extr. no. 2	16.50—17.05	15	0			
21.	»	17.05—17.20	15	0.4	congo pos.		»
22.	Injection of p. extr. no. 2 + stimul. of vagi	17.20—17.35	15	3.0	45.5	55.0	»
23.	Injection of p. extr. no. 2	17.35—17.50	15	1.5	congo pos.		»

TABLE XVIII.

Cat 3.4 kg. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extract from pig's pyloric mucosa was injected intravenously. The vagi were concomitantly stimulated. Conc. of extract; 0.25 gm. moist mucosa/cc. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion	
		hour	min.	volume cc.	acidity
1.	Stimul. of vagi	11.05—11.20	15	1.8	congo pos.
2.	»	11.20—11.35	15	1.3	»
3.	»	11.35—11.50	15	2.2	»
4.	»	11.50—12.05	15	2.0	»
5.	No stimulation	12.05—12.20	15	1.3	»
6.	»	12.20—12.35	15	0.7	»
	Pyloric mucosa cocainized	12.35—12.55	20		
	No stimulation	12.55—13.20	25		
7.	Stimul. of vagi	13.20—13.35	15	3 drops	congo neg.
8.	»	13.35—13.50	15	3 »	»
9.	Injection of p. extr. + stimul. of vagi	13.50—14.05	15	2 »	»
10.	»	14.05—14.20	15	2.1	congo pos.
11.	»	14.20—14.35	15	1.1	»
12.	»	14.35—14.50	15	1.2	»

TABLE XIX.

Cat 4.5 kg. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Following this, extract from cat's pyloric mucosa was injected intravenously; during definite periods the vagi were concomitantly stimulated. Conc. of extract: 0.5 gm moist mucosa/cc. Histamine content 0.01 γ/cc. Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Gastric content	11.10		7.8	0.5	10.0
2.	No stimulation	11.10—11.25	15	0.5	congo neg.	
3.	Stimul. of vagi	11.25—11.40	15	5.2	64.5	70.5
4.	»	11.40—11.55	15	10.2	145.5	149.5
5.	»	11.55—12.10	15	7.2	150.5	155.5
6.	No stimulation	12.10—12.40	30	1.8	congo pos.	
	Pyloric mucosa cocainized (3 %)	12.40—12.55	15			
7.	No stimulation	12.55—13.25	30	0		
8.	Stimul. of vagi	13.25—13.40	15	a few drops	congo neg.	
9.	»	13.40—13.55	15	0.3	»	
10.	Injection of p. extr. + stimul. of vagi	13.55—14.10	15	0.5	»	
11.	»	14.10—14.25	15	4.8	83.0	92.5
12.	Stimul. of vagi	14.25—14.40	15	3.5	130.0	134.5
13.	»	14.40—14.55	15	0.5	congo pos.	
14.	No stimulation	14.55—15.25	30	a few drops	»	
15.	Injection of p. extr.	15.25—15.40	15	»	»	
16.	»	15.40—15.55	15	0.3	»	
17.	Injection of p. extr. + stimul. of vagi	15.55—16.10	15	0.2	»	
18.	»	16.10—16.25	15	2.6	»	
19.	»	16.25—16.40	15	4.3	118.0	131.0
20.	»	16.40—16.55	15	3.9	142.5	149.0
21.	Stimul. of vagi	16.55—17.10	15	1.4	congo pos.	
22.	»	17.10—17.25	15	1.1	»	
23.	»	17.25—17.40	15	0.7	»	

TABLE XX.

Dog 12 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Following this, extracts from canine pyloric mucosa were injected intravenously. During definite periods the vagi were concomitantly stimulated.

Conc. of extracts (gm. moist mucosa/cc): p. extr. no. 1 0.2 gm./cc.; p. extr. no. 2 0.3 gm./cc. Histamine content: p. extr. no. 1 0.08 γ/cc.; p. extr. no. 2 0.03 γ/cc. Intensity of stimulus 3 m. a.

Sample	Procedure	Time		Gastric Secretion			
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.	character of juice
1.	Gastric content	11.15		67.0	35.5	45.5	watery
2.	No stimulation	11.15—11.30	15	4.5	40.0	49.0	»
3.	Stimul. of vagi	11.30—11.45	15	17.5	68.0	76.0	»
4.	»	11.45—12.00	15	6.5	123.5	130.0	»
5.	»	12.00—12.15	15	9.5	111.0	116.0	»
6.	»	12.15—12.30	15	3.0	100.5	107.0	»
7.	»	12.30—12.45	15	9.5	115.0	119.5	»
	Resection of pyloric region	12.45—13.30	45				
8.	No stimulation	13.30—14.00	30	0			
9.	Stimul. of vagi	14.00—14.15	15	0.6	congo pos.		
10.	»	14.15—14.30	15	3.5	0	pH 6.4	stringy, mucous
11.	»	14.30—14.45	15	3.5	0	pH 7.8	»
12.	»	14.45—15.00	15	3.0	0	pH 8.0	»
13.	No stimulation	15.00—15.30	30	0			
14.	Injection of p. extr. no. 1	15.30—15.45	15	0.3	congo neg.		»
15.	»	15.45—16.00	15	0.4	»		»
16.	Injection of p. extr. no. 1 + stimul. of vagi	16.00—16.15	15	3.5	congo first neg.; after more and more pos.		first mucous; after more and more watery

Table XX (Cont.).

Sample	Procedure	Time: hour	Time: min.	Gastric Secretion: volume cc.	Gastric Secretion: free HCl m. eq./lit.	Gastric Secretion: total HCl m. eq./lit.	Gastric Secretion: character of juice
17.	Injection of p. extr. no. 1 + stimul. of vagi	16.15—16.30	15	3.5	21.5	42.0	somewhat mucous
18.	Stimul. of vagi	16.30—16.45	15	5.5	53.0	67.0	»
19.	»	16.45—17.00	15	3.0	congo pos.		more mucous
20.	»	17.00—17.15	15	4.0	»		stringy, mucous
21.	No stimulation	17.15—17.45	30	0.4	»		mucous
22.	Injection of p. extr. no. 2	17.45—18.00	15	0.8	»		»
23.	»	18.00—18.15	15	4.0	60.5	76.0	less mucous
24.	Injection of p. extr. no. 2 + stimul. of vagi	18.15—18.30	15	15.5	64.5	75.5	watery
25.	No stimulation	18.30—18.45	15	2.0	congo pos.		»
26.	»	18.45—19.00	15	0.2	»		

TABLE XXI.

Cat 3.8 kg. Gastric secretion during stimulation of vagi before and after cocainization of pyloric mucosa. Followin this, extracts from cat's pyloric and corpus mucosa were injected intravenously; during definite periods the vagi were concomitantly stimulated.

Conc. of extracts (gm. moist mucosa/cc.): p. extr. 0.2 gm./cc.; c. extr. 0.1 gm./cc. Histamine content of p. extr. 0.1 γ/cc. Intensity of stimulus 1 m. a.

Sample	Procedure	Time: hour	Time: min.	Gastric Secretion: volume cc.	Gastric Secretion: free HCl m. eq./lit.	Gastric Secretion: total HCl m. eq./lit.
1.	Gastric content	12.05		2.9	0	8.0
2.	No stimulation	12.05—12.20	15	0.6	congo neg.	
3.	Stimul. of vagi	12.20—12.35	15	3.3	26.5	36.5
4.	»	12.35—12.50	15	5.2	110.0	117.5
5.	»	12.50—13.05	15	2.6	120.0	128.0
6.	»	13.05—13.20	15	2.0	96.0	103.0
	Pyloric mucosa cocainized (2 %)	13.20—13.50	30			
7.	No stimulation	13.50—14.20	30	0		
8.	Stimul. of vagi	14.20—14.35	15	0.2	congo neg.	
9.	»	14.35—14.50	15	0.4	»	
10.	Injection of p. extr. + stimul. of vagi	14.50—15.05	15	2.9	37.5	44.0
11.	Stimul. of vagi	15.05—15.20	15	6.6	113.0	117.5
12.	»	15.20—15.35	15	0.9	congo pos.	
13.	Injection of p. extr.	15.35—15.50	15	1.6 }	97.5	104.5
14.	No stimulation or injection	15.50—16.05	15	2.3 }		
15.	Injection of p. extr. + stimul. of vagi	16.05—16.20	15	0.8	congo pos.	
16.	»	16.20—16.35	15	7.0	125.0	129.0
17.	Stimul. of vagi	16.35—16.50	15	6.7	132.5	136.5
18.	»	16.50—17.05	15	1.9	congo pos.	
19.	Injection of c. extr. + stimul. of vagi	17.05—17.20	15	1.5 }		
20.	»	17.20—17.35	15	1.3 }	75.0	83.0
21.	»	17.35—17.50	15	1.1 }		

TABLE XXII.

Cat 3.5 kg. Oesophagus tied in neck. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Following this, extracts from cat's pyloric and corpus mucosa were injected intravenously; during definite periods the vagi were concomitantly stimulated.

Conc. of extracts (gm. moist mucosa/cc.): p. extr. 0.25 gm./cc.; c. extr. 0.5 gm./cc. Histamine content: p. extr. 0.12 γ/cc. c. extr. 0.03 γ/cc.

Intensity of stimulus 1 m. a.

Sample	Procedure	Time		Gastric Secretion		
		hour	min.	volume cc.	free HCl m. eq./lit.	total HCl m. eq./lit.
1.	Gastric content	11.00		3.8	25.0	55.0
2.	Stimul. of vagi	11.00—11.15	15	3.8	50.0	90.0
3.	»	11.15—11.30	15	3.8	78.5	102.0
4.	»	11.30—11.45	15	3.2	congo pos.	
5.	No stimulation	11.45—12.00	15	2.0	67.0	97.0
6.	»	12.00—12.15	15	0.6	congo pos.	
	Resection of pyloric region	12.15—12.30	15			
7.	No stimulation	12.30—13.00	30	0		
8.	Stimul. of vagi	13.00—13.15	15	1 drop	congo pos.	
9.	»	13.15—13.30	15	0.6 }	»	
10.	»	13.30—13.45	15	0.7 }	»	
11.	Injection of p. extr. + stimul. of vagi	13.45—14.00	15	1.2	congo pos.	
12.	»	14.00—14.15	15	3.0	84.0	105.0
13.	No stimulation	14.15—14.30	15	2.3	91.0	109.5
14.	»	14.30—14.45	15	0.3	congo pos.	
15.	»	14.45—15.00	15	0		

Table XXII (Cont.)

Sample	Procedure	Time: hour	Time: min.	Gastric Secretion: volume cc.	Gastric Secretion: free HCl m. eq./lit.	Gastric Secretion: total HCl m. eq./lit.
16.	Injection of p. extr.	15.00—15.15	15	0		
17.	»	15.15—15.30	15	0.7	congo pos.	
18.	»	15.30—15.45	15	1.2	»	
19.	Injection of p. extr. + stimul. of vagi	15.45—16.00	15	2.7	89.0	107.0
20.	»	16.00—16.15	15	3.8	102.0	114.0
21.	Stimul. of vagi	16.15—16.30	15	2.9	111.0	122.5
22.	»	16.30—16.45	15	1.6	107.0	112.0
23.	»	16.45—17.00	15	1.5		
24.	Injection of c. extr. + stimul. of vagi	17.00—17.15	15	1.5		
25.	»	17.15—17.30	15	1.2	102.0	113.0
26.	»	17.30 —17.45	15	0.8		
27.	Stimul. of vagi	17.45—18.00	15	0.9	congo pos.	
28.	»	18.00 —18.15	15	0.7		

TABLE XXIII.

Cat 3.3 kg. Gastric secretion during intravenous injection of histamine before and after resection of pyloric region.

Sample	Procedure	Time: hour	Time: min.	Gastric Secretion: volume cc.	Gastric Secretion: free HCl m. eq./lit.	Gastric Secretion: total HCl m. eq./lit.
1.	Gastric content	10.00		2.1	42.5	66.0
2.	Injection of histamine 5 γ/kg./min.	10.15—10.30	15	0.5	congo pos.	
3.	»	10.30—10.45	15	1.5	»	
4.	»	10.45—11.00	15	1.0	»	
5.	»	11.00—11.15	15	0.9		
6.	»	11.15—11.30	15	0.7		
7.	Injection of histamine 10 γ/kg./min.	11.30—11.45	15	1.0	86.5	97.0
8.	»	11.45—12.00	15	0.6		
9.	»	12.00—12.15	15	0.7		
10.	»	12.15—12.30	15	1.2		
11.	»	12.30—12.45	15	1.0	79.0	95.0
12.	Injection of histamine 20 γ/kg./min.	12.45—13.00	15	0.9		
13.	»	13.00—13.15	15	2.4		
14.	»	13.15—13.30	15	4.2		
15.	»	13.30—13.45	15	4.4		
16.	»	13.45—14.00	15	6.0	116.0	120.5
17.	»	14.00—14.15	15	6.6		
18.	No injection	14.15—14.30	15	4.4	120.0	122.0
	Resection of pyloric region	14.30—15.30	60			
19.	Injection of histamine 20 γ/kg./min.	15.30—15.45	15	0.5		
20.	»	15.45—16.00	15	2.8		
21.	»	16.00—16.15	15	5.3	110.0	116.0
22.	»	16.15—16.30	15	5.0		
23.	»	16.30—16.45	15	5.8	125.5	128.5
24.	»	16.45—17.00	15	5.0		
25.	No injection	17.00—17.15	15	2.3		
26.	»	17.15—17.30	15	1.5		

TABLE XXIV.

Cat 3.3 kg. Gastric secretion during stimulation of vagi before and after resection of pyloric region. Histamine was slowly injected intravenously; the vagi were stimulated during later stage of injection. Intensity of stimulus 1 m. a.

Sample	Procedure	Time hour	Time min.	Gastric Secretion volume cc.	Gastric Secretion acidity
1.	Gastric content	11.00		1.1	
2.	No stimulation	11.00—11.15	15	0	
3.	Stimul. of vagi	11.15—11.30	15	0.8	congo pos.
4.	»	11.30—11.45	15	1.0	»
5.	»	11.45—12.00	15	1.5	»
6.	»	12.00—12.15	15	2.1	»
7.	»	12.15—12.30	15	2.1	»
	Resection of pyloric region	12.30—13.00	30		
8.	No stimulation	13.00—13.15	15	0	
9.	Stimul. of vagi	13.15—13.30	15	0.2	congo faintly pos.
10.	»	13.30—13.45	15	0.4	congo pos.
11.	»	13.45—14.00	15	0.7	»
12.	Injection of histamine 1 γ/kg./min.	14.00—14.15	15	0.2	»
13.	»	14.15—14.30	15	0.2	»
14.	»	14.30—14.45	15	0.4	»
15.	»	14.45—15.00	15	0.3	»
16.	Injection of histamine 1 γ/kg./min. + stimul. of vagi	15.00—15.15	15	0.5	»
17.	»	15.15—15.30	15	0.7	»
18.	»	15.30—15.45	15	0.5	»